IJARBN

ISSN: 2582-3310

July 2022, Vol. 3, Issue I

International Journal of Advanced Research in Biotechnology and Nanobiotechnology

Published By:

Amity Institute of Biotechnology

Amity University Madhya Pradesh, Gwalior

International Journal of Advanced Research in Biotechnology and Nanobiotechnology
Vol. 3, Issue I, July 2022 **ISSN: 2582-3310**

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Antimicrobial and Preservative property of Pomegranate: Review

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ABSTRACT

The pomegranate has been known for hundreds of years for its multiple health benefits. The fruit is very rich in nutrition, and in addition to that, it has antimicrobial and preservative properties. It has indestructible anti-bacterial activity against a wide range of pathogenic bacteria such as *Salmonella typhi, Vibrio cholera, Shigella* sp., *Escherichia coli,* and *Listeria monocytogenes.* It inhibits several fungi like *Rhizoctonia solani, Aspergillus niger, Saccharomyces cerevisiae*, etc.

This review aims to exploit the medicinal value of pomegranates in treating various diseases and the antioxidant potential of the active ingredient in pomegranate extracts.

An extensive and systemic review of studies was carried out, and it reveals that the fruit and their extracts may act as a natural alternative to various types of bacterial, viral, and fungal pathogens and have preservative potential in food items like PEP, which was used in cream preservation, cheese preservation, and beef burger preservation. Each and every part of the pomegranate plant is trialled for its bactericidal activity, including leaves, peel, bark, and juice, many studies reveal success with peel extract as it has a high level of antioxidants.

The objective of the present study is to evaluate the antimicrobial activity of different parts of the pomegranate. Several projects have also been done that show the medicinal value of the pomegranate part, and the current studies do support the potential benefit of it. An integrated approach is needed to verify the medicinal and preservative use of pomegranates on different species of microbes and to understand the effect of extracts on different human diseases and also to see its antimicrobial activities on humans.

Key word: *Punica granatum*, antibacterial activity, antimicrobial activity, pomegranate peel extract (PEP), antioxidants.

INTRODUCTION

Punica granatum has its own vernacular name, pomegranate. Pomegranates are a popular table fruit in Asia, especially in the Pacific and Indian regions. According to the agriculture exchange government of India, out of all 5 continents, the Asia-Pacific region leads the market for pomegranates. Every year, the production of pomegranates increases by 20–25%. The fruit is mostly cultivated in north India, Iran, China, and the Mediterranean region [1]. The fruit has a very high level of antioxidants, which helps in reducing free radicals from the body and has been traditionally used for diarrhoea, dysentery, etc since ancient times [2]. Strong bactericidal activity has also been observed against several pathogenic fungi and bacteria. Bacteria which are inhibited by pomegranate peel extract (POPx) are *Salmonella typhi, Vibrio cholera, Escherichia coli, Staphylococcus aureus,* etc [3-4]. The fungus species which are inhibited by hydro-alcoholic POPx are dermatophyte species (trichophyton, epidermophyton, and micosporum) [5].

In terms of therapeutic activity, pomegranate rind methanolic extract is found to have anti-inflammatory and anti-allergic properties. [6]. There is also evidence of pomegranates' ability to control viruses affecting the human body. Most of the viruses are those that are food borne. These viruses are the Aichi virus, hepatitis E virus, hepatitis A virus, rotavirus, human norovirus, and small round-structured virus, along with other human enteroviruses [7]. Of all these viruses, the human norovirus is a major cause of viral gastroenteritis [8–10].

A 

B 

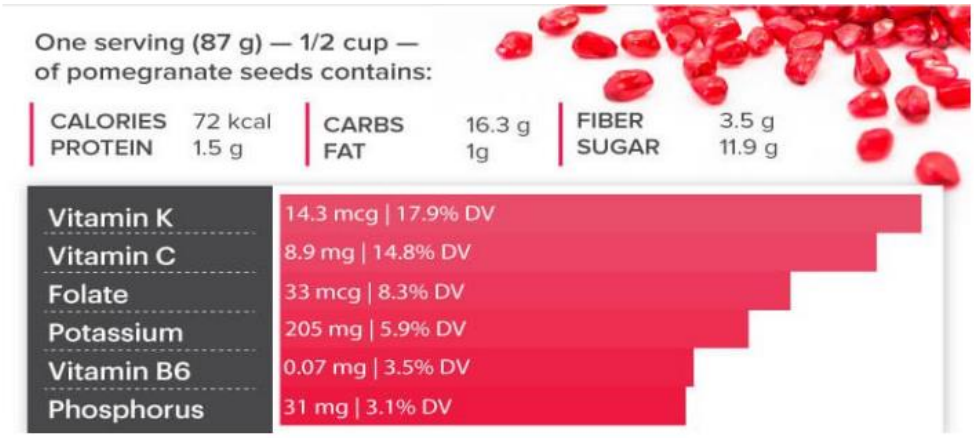
C 

D 

Figure 1; A) Pomegranate fruit; B) Pomegranate peel powder; C) Pomegranate seed; D) Pomegranate peel dried in hot air oven.

MEDICINAL PROPERTY

Pomegranates have traditionally been used as medicine for diseases like diarrhoea, helminthiasis, dysentery, and respiratory pathology [11-14]. Pomegranates have been extensively identified for their antimicrobial activity and therapeutic potential. Due to the abundance of antioxidants in pomegranates, they are medicinally used [15]. Pomegranates are rich in different vitamins, such as vitamin K and vitamin C. In the case of vitamin C, it helps in repairing all body tissue and its development. In daily life, we need 65-90mg of vitamin C. Pomegranates contain 30 mg of vitamin C, which is about 50% of the daily intake recommendation. Whereas in the case of vitamin K, it helps to produce various types of proteins that are required for bone building and blood clotting (helps in the thickening of blood and stops bleeding naturally).



POMEGRANATE AND THIER EFFECT ON HUMAN BACTERIA

Antibacterial use of pomegranates has a long history since biblical times. Pomegranates were used by the Egyptians to treat a number of different infections. Traditionally, it was used in the Ayurvedic system of medicine. An extract from pomegranate fruit and the bark of a tree is used for diarrhoea and dysentery. There are several projects that have also been done globally on the antibacterial activity of pomegranates. On highly pathogenic and drug-resistant strains, bactericidal activity was investigated. All these different studies are done to determine the

bactericidal potency of different extracts of pomegranate plants against a different range of bacteria using different types of methods like minimum inhibitory concentration (MIC) and the disc diffusion method. The effect of pomegranates on different bacteria is shown in table [37].

Bacteria	Pomegranate extract	Growth inhibition (–) or promotion (+)
Enteric		
Escherichia coli O157:H7	Peel, bark	–
Salmonella Typhi	Peel	–
Salmonella Typhimurium	Peel	–
Salmonella enterica serovars	Peel	
Vibrio cholerae	Peel	–
Yersinia enterocolitica	Peel	–
Shigella spp.	Peel	–
Shigella sonnei	Peel	–
Listeria monocytogenes	Peel, dried juice powder	–
Staphylococcus aureus	Peel, juice, and POMx	–
Clostridium spp.	POMx	
Probiotic		
Bifidobacterium spp.	POMx	+
Lactobacillus spp.	POMx	+
Bifidobacterium breve	POMx	+
Bifidobacterium infantis	POMx	+
Wound		
Pseudomonas aeruginosa	Peel, flower extract	–
Staphylococcus aureus	Peel	–
Escherichia coli	Peel	–
Klebsiella pneumoniae	Peel	–
Salmonella Anatum	Peel	–
Salmonella Typhimurium	Peel	–
Streptococcus pneumoniae	Peel	–
Oral		
Staphylococcus aureus	Peel	–
Staphylococcus epidermidis	Peel	–
Streptococcus mutans	Peel	–
Streptococcus salivarius	Peel	–
Streptococcus sanguis	Peel	–
Streptococcus mitis	Peel	–
Porphyromonas gingivalis	Peel	–
Aggregatibacter actinomycetemcomitans	Peel	–
Prevotella intermedia	Peel	–
Proteus spp.	Peel	–
Drug resistant		
Methicillin-resistant *Staphylococcus aureus*	Peel	–
Acinetobacter baumannii	Peel	–
Helicobacter pylori	Peel	–

TABLE: 1 (POMx =Pomegranate fruit extract)

ANTIBACTERIAL ACTIVITY OF POMEGRANATE

In Thailand, a study was conducted on three different strains of *Escherichia coli*, and it has been found that the pomegranate shows high bactericidal activity. The pomegranate ethanolic extract shows strong inhibition of the *E. coli O157:h7 strain (bacteriostatic as well as bactericidal).* As a result, infection caused by the e.coli O157:h7 strain can be controlled by an ethanolic extract of pomegranate [16-17].

Pomegranate antibacterial activity is found against different pathogenic waterborne bacteria, *including salmonella typhi, vibrio cholera, yersinia entrocolitica, shigella spp., and listeria monocytogenes* [18].

Among several bacterial diseases, typhoid is the most deadly enteric fever that causes several deaths per year. It is caused by bacteria, which easily enters the human body through contaminated food or contaminated water. Pomegranate fruit peel (pericarp) extract has been tested with it and it inhibits the growth of bacteria [19]. Methanolic extract of pomegranate peel exhibits high bactericidal activity against *vibrio cholerae* [20].

Another study shows that pomegranate peel extract infused with tea was effective against Shigella spp. (cause of diarrhoea) [21]. The effects of different concentrations of methanolic pomegranate peel extract at 12 mg/ml, 8 mg/ml, and 4 mg/ml were observed on the growth of dental bacteria and were compared using the disc diffusion method. All of the three concentrations show bactericidal activity against *staphylococcus epidermis* and *staphylococcus aureus.* Extracts with concentrations of 12 mg/ml and 8 mg/ml show bactericidal effects against *lactobacillus acidophilus, streptococcus mutans,* and *streptococcus salivarius,* but all concentrations are unable to inhibit the *actinomyces viscosus.* [22]

ANTIVIRAL ACTIVITY OF POMEGRANATE

There are a very limited number of studies that have been done on the antiviral activity associated with pomegranates and their different extracts. Pomegranate fruit has been reported to have antiviral activity against the human immunodeficiency virus (HIV), influenza virus, and pox virus [23–25]. Anthocyanins and the hydrolyzable tannins are the major elements associated with the antiviral activity of pomegranates. In one study on the antiviral activity of pomegranates, it was discovered that only punicalagin, one of the four flavonoid compounds, has an antiviral effect on the influenza virus [26]. Increased research and the need for alternative natural antivirals with no

side effects is required, as it is recorded that the toxic effects of the recommended doses of medicine are high, so the pomegranate can serve as an additional beneficial drug with no side effects. Therefore, pomegranate juice and pomegranate extract could possibly be used to inhibit various types of viruses that are transmitted via infected food products and through body fluids.

A study found that pomegranate extract has polyphenols, which are antiviral agents for the influenza virus. Using real-time polymerase chain reaction, a virus prevents virus-induced agglutination of chicken red blood cells and suppresses virus replication in the host [23]. Among all four polyphenols of pomegranate, punicalagin is the most effective anti-influenza component, which inhibits the replication of RNA of the influenza virus and also inhibits the agglutination of chicken red blood cells by the viruses. To understand the mechanism of action of human noroviruses, the monolayer of the host cell for the respective virus was treated with polyphenols and pomegranate juice during or after infection, where infectivity of (FCV-F9) feline calcivirus and (MNV-1) murine norovirus was obtained to be suppressed [27]. Pomegranate juice and pomegranate extracts were found to have a much larger effect on the life cycle reduction of viruses when the treatment was performed prior to the infection than after infection (corresponds to the replication stage). It suggests that pomegranate juice and polyphenols may have a role in suppressing/blocking virus binding to the host cell receptor or by covering the surface of the cell where receptors are present or by blocking the ligands on the virus surface. With the help of a transmission type electron microscope (TEM), it has been theorized that we may find out whether polyphenols cause structural or functional damage to the virus or not. Researchers [24] discovered that HIV-1 entry inhibitors derived from pomegranate juice are adsorbed onto corn starch, where they block HIV-1 binding to CD4 and host cell receptors, limiting infection by primary viruses with similar genetic codes. These researchers found the potential to produce anti-HIV-1 from naturally safe food products like pomegranates.

Phenolic compounds of fruit.

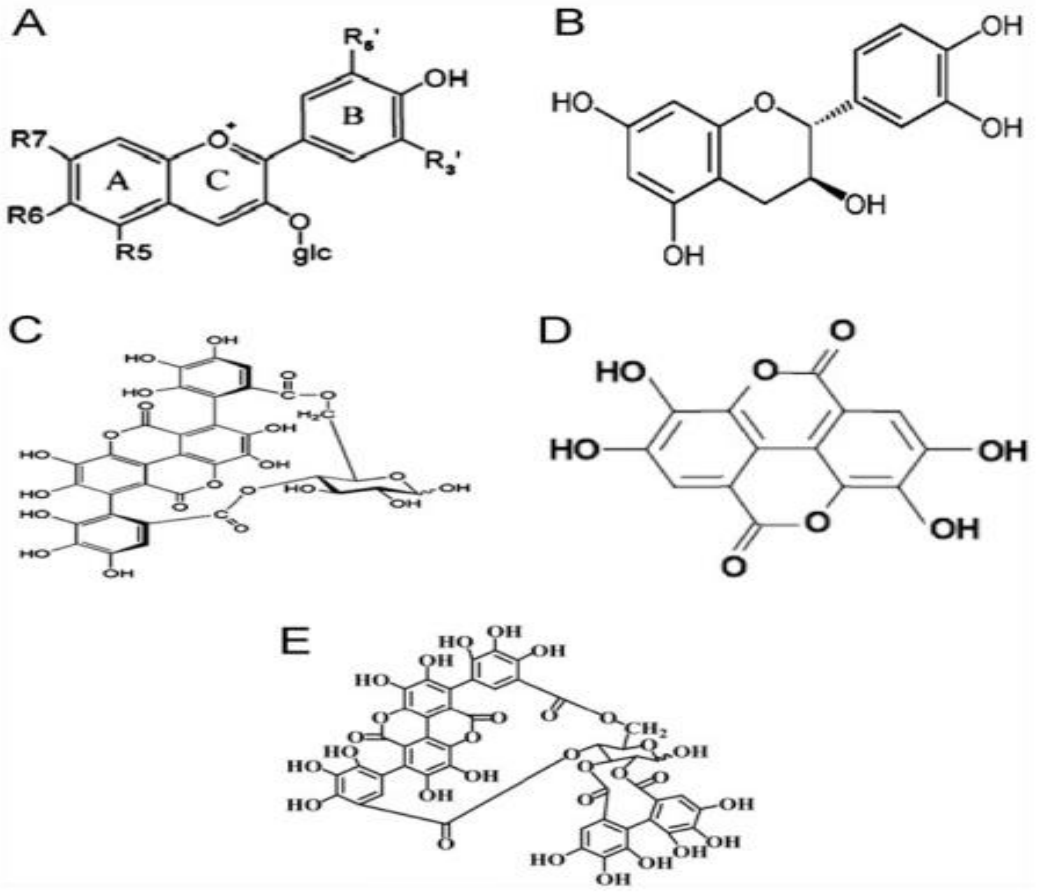


Fig. 2. General structure of the important phenolic compounds of pomegranate peel (A) Anthocyanin (B) Catechins (C) Punicalin (D) Elagic acid (E) Punicalagin.

THE ANTI-FUNGAL PROPERTY OF POMEGRANATES

According to a study conducted by some researchers, pomegranates have antifungal activity against candida species, and the peel of pomegranates is most effective against candida albicans [28-29].explains that pomegranate peel extract had a strong inhibition against *candida truncatum, candida coccodes, and rhizoctonia solani. Fusarium solani and Alternaria alternate* are unaffected [30].

Dermatophyte species like *Dermatophyte epidermophyton, Dermatophyte trichophyton, and Dermatophyte microsporum* are the species that infect the epidermis and appendages with serious health consequences. The dermatophyte fungi trichophyton mentagrophytes, trichophyton rubrum, *microsporum canis*, and *microsporum gypseum* were inhibited by the hydro-alcholic pomegranate peel extract. *Trichophyton mentagrophytes*, Trichophyton rubrum, *Microsporum canis*, and *Microsporum gypseum* are dermatophyte fungi. Punicalagin and crude extract show antifungal activity on two growth phases of fungi, i.e., on the hyphal stage and the conidial stage. In reference

to mammalian cells, the cyto-toxicity assay is specifically for fungal cells or fungal culture. From an overview of the studies, it is concluded that crude extract and punicalagin indicate high antifungal activity against trichophyton rubrum. Therefore, it is possible to make an anti-fungal medicine with pomegranates and their extracts. [31].

ANTI-INFLAMMATORY AND ANTI-ALLERGIC EFFECT OF POMEGRANATE

It has been found the therapeutic benefit of pomegranates is of high value. Its fraction has built a scientific consensus that pomegranate rind methanolic extract has the ability to inhibit inflammation and allergies [9]. The anti-inflammatory components of pomegranate peel that are strictinin A, punicalagin, granatum B, and punicalin have a tendency to reduce nitric oxide by inhibiting the expansion of pro-inflammatory proteins [32–33]. Evidently, inflammatory cells including neutrophils, monocytes and macrophages may inflict damage to nearby tissue, an event thought to be of phatogenic significance in a large number of diseases such as acute respiratory distress syndrome, arthero-sclerosis, emphysema, reperfusion injury, rheumatoid arthritis and malignancy [34].

PRESERVATIVE PROPERTY OF POMEGRANATE

As it is known, pomegranates have antifungal activity. It inhibits several fungi like *Rhizoctonia solani, Aspergillus niger, Saccharomyces cerevisiae, etc.* These species of fungus affect several food products and can be controlled by PEP. In research with cream preservation, when PEP was added at 1% level of fat, research was conducted to observe the preservative potential. Shelf life of cream was found to be one month at a refrigeration temperature [35]. In other research, researchers found that, in beef burgers, when pomegranate peel powder was added, the beef was found to have a longer shelf life at refrigeration temperature as compared to beef where no pomegranate powder was added into the beef burger. Prepared beef burger samples containing pomegranate peel powder recorded high cooking quality in comparison to control beef burger samples. The use of pomegranate peel powder at ratios of 1, 2, and 3% has proved to be effective as a natural preservative in producing high quality beef burger samples. The addition of different concentrations of pomegranate peel powder improved the aforementioned quality criteria. The application of different ratios of pomegranate peel powder has improved the cooking characteristics, e.g., cooking loss, cooking yield, and change in diameter. At the same time, the

tested ratios of pomegranate peel powder could be useful to achieve high stability of beef burgers during refrigerated storage, with positive effects on the sensory characteristics of the product. [36].

CONCLUSION

Pomegranate peel, which is basically a waste for almost all of us, can be used for medicinal purposes. Large number of research has been done on fruits. The anti-microbial activity of pomegranates is found in almost every part of them. The pomegranate peel shows the high value of antioxidants. From this point of view, pomegranates have wide acceptance in medicinal and pharmacological use against different bacterial diseases, viral diseases, fungal diseases, allergies, inflammation, and digestive disorders. It can be used as a natural preservative too.

The high medicinal use of this fruit shows its importance in preventing living beings from various diseases. Therefore, pomegranates are a good target to study and to obtain a new medicine and a preservative from them.

Famous Hindi idiom "एक अनार सौ बीमार" explains it all.

Hence it can be very useful in the future for the cure of various diseases

REFERENCE:

1. https://agriexchange.apeda.gov.in/Weekly_eReport/Pomegranate_Report.pdf
2. Lansky, E., Shubert, S., Neeman, I., 2004. Pharmacological and therapeutic properties of pomegranate. CIHEAM–Options Mediterraneennes xx, 231–235.
3. S. S. Dahham, M. N. Ali, and H. Tabassum, "Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.)," American-Eurasian Journal of Agricultural & Environmental Sciences, vol. 9, no. 3, pp. 273–281, 2010.
4. T. Ismail, P. Sestili, and S. Akhtar, "Pomegranate peel and fruit extracts: a review of potential antiinflammatory and anti-infective effects," Journal of Ethnopharmacology, vol. 143, no. 2, pp. 397–405, 2012.
5. Rinaldi MG: Dermatophytosis: Epidemiological and microbiological, update. J Am Acad Dermatol 2000, 43:120–124.
6. Panichayupakaranant, P., Tewtrakul, S., Yuenyongsawad, S., 2010b. Antibacterial, antiinflammatory and antiallergic activities of standardized pomegranate rind extract. Food Chemistry 123, 400–403.

7. D. H. D'Souza, A. Sair, K. Williams et al., "Persistence of caliciviruses on environmental surfaces and their transfer to food," International Journal of Food Microbiology, vol. 108, no. 1, pp. 84–91, 2006.
8. E. Scallan, R. M. Hoekstra, F. J. Angulo et al., "Foodborne illness acquired in the United States-Major pathogens," Emerging Infectious Diseases, vol. 17, no. 1, pp. 7–15, 2011.
9. R. M. Turcios, M. A. Widdowson, A. C. Sulka, P. S. Mead, and R. D. Glass, "Reevaluation of epidemiological criteria for identifying outbreaks of acute gastroenteritis due to norovirus: United States, 1998–2000," Clinical Infectious Diseases, vol. 42, no. 7, pp. 964–969, 2006.
10. J. J. Siebenga, H. Vennema, D. P. Zheng et al., "Norovirus illness is a global problem: emergence and spread of norovirus gii.4 variants, 2001–2007," Journal of Infectious Diseases, vol. 200, no. 5, pp. 802–812, 2009.
11. Braga LC, Shupp JW, Cummings C, Jett M, Takahashi JA, Carmo LS, et al. Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production.J Ethnopharmacol 2005; 96: 335-9.
12. Choi JG, Kang OH, Lee YS, Chae HS, Oh YC, Brice OO, et al. In vitro and in vivo antibacterial activity of Punica granatum peel ethanol extract against salmonella. Evid Based Complement Alternat Med 2011; 2011: 690518.
13. Reddy MK, Gupta SK, Jacob MR, Khan SI, Ferreira D.Antioxidant, antimalarial and antimicrobial activities of tannin- rich fractions, ellagitannins and phenolic acids from Punica granatum L. Planta Med 2007; 73: 461-7
14. Sa´nchez-Lamar A, Fonseca G, Fuentes JL, Cozzi R, Cundari E, Fiore M, et al. Assessment of the genotoxic risk of Punicabgranatum L. (Punicaceae) whole fruit extracts. J Ethnopharmacol 2008; 115: 416-22.
15. T. Ismail, P. Sestili, and S. Akhtar, "Pomegranate peel and fruit extracts: a review of potential antiinflammatory and anti-infective effects," Journal of Ethnopharmacology, vol. 143, no. 2, pp. 397–405, 2012.
16. Voravuthikunchai, A. Lortheeranuwat, W. Jeeju, T. Sririrak, S. Phongpaichit, and T. Supawita, "Effective medicinal plants against enterohaemorrhagic Escherichia coli O157:H7," Journal of Ethnopharmacology, vol. 94, no. 1, pp. 49–54, 2004.

17. S. P. Voravuthikunchai and S. Limsuwan, “Medicinal plant extracts as anti-Escherichia coli O157:H7 agents and their effects on bacterial cell aggregation,” Journal of Food Protection, vol. 69, no. 10, pp. 2336–2341, 2006.
18. Amy B.howell and Doris H. D'souza. The pomegranate : effect on bacteria and virus that influence human health. Vol 2013 Article ID 606212
19. C. Pérez and C. Anesini, “In vitro antibacterial activity of Argentine folk medicinal plants against Salmonella typhi,” Journal of Ethnopharmacology, vol. 44, no. 1, pp. 41–46, 1994.
20. J. M. Guevara, J. Chumpitaz, and E. Valencia, “The in vitro action of plants on Vibrio cholerae,” Revista de Gastroenterologia del Peru, vol. 14, no. 1, pp. 27–31, 1994.
21. A. D. Alanís, F. Calzada, J. A. Cervantes, J. Torres, and G. M. Ceballos, “Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders,” Journal of Ethnopharmacology, vol. 100, no. 1-2, pp. 153–157, 2005.
22. S. Abdollahzadeh, R. Y. Mashouf, H. Mortazavi et al., “Antibacterial and antifungal activities of Punica granatum peel extracts against oral pathogens,” Journal of Dentistry, vol. 8, no. 1, pp. 1–6, 2011.
23. M. Haidari, M. Ali, S. W. Casscells, and M. Madjid, “Pomegranate (Punica granatum) purified polyphenol extract inhibits influenza virus and has a synergistic effect with oseltamivir,” Phytomedicine, vol. 16, no. 12, pp. 1127–1136, 2009.
24. R. Neurath, N. Strick, Y. Y. Li, and A. K. Debnath, “Punica granatum (pomegranate) juice provides an HIV1 entry inhibitor and candidate topical microbicide,” in Natural Products and Molecular Therapy, G. J. Kotwal and D. K. Lahiri, Eds., vol. 1056, pp. 311–327, New York Academy of Sciences, 2005
25. G. J. Kotwal, “Genetic diversity-independent neutralization of pandemic viruses (e.g. HIV), potentially pandemic (e.g. H5N1 strain of influenza) and carcinogenic (e.g. HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCs),” Vaccine, vol. 26, no. 24, pp. 3055–3058, 2008.
26. S. G. Kasimsetty, D. Bialonska, M. K. Reddy, C. Thornton, K. L. Willett, and D. Ferreira, “Effects of pomegranate chemical constituents/intestinal microbial metabolites on CYP1B1 in 22Rv1 prostate cancer cells,” Journal of Agricultural and Food Chemistry, vol. 57, no. 22, pp. 10636–10644, 2009.

27. X. Su, M. Y. Sangster, and D. H. D'Souza, "in vitro effects of pomegranate juice and pomegranate polyphenols on foodborne viral surrogates," Foodborne Pathogens and Disease, vol. 7, no. 12, pp. 1473– 1479, 2010.
28. A.A. Tayel, W.F. El-Tras. Mycoses 53, 117-122 (2009).
29. H. Endo, D.A.G. Cortez, T. Ueda-Nakamura, C.V. Nakamura, B. P. D. Filho. Res Microbiol. 161,534-540 (2010).
30. E. Osorio, M. Flores, D. Hernández, J. Ventura, R. Rodríguez, CN. Aguilar. Ind. Crop Prod. 31(1),153-157 (2010).
31. Simone R Foss, Celso V Nakamura, Tania Ueda-Nakamura, Diógenes AG Cortez, Eliana H Endo and Benedito P Dias Filho. Anti fungal activity of pomegranate peel extract and isolated compound punicalagin against dermatophytes
32. Lee, S.I., Kim, B.S., Kim, K.S., Lee, S., Shin, K.S., Lim, J.S., 2008. Immune-suppressive activity of punicalagin via inhibition of NFAT activation. Biochemical and Biophysical Research Communications 371, 799–803.
33. Romier, B., Van De Walle, J., During, A., Larondelle, Y., Schneider, Y.J., 2008.Modulation of signalling nuclear factor-kappaB activation pathway by polyphenols in human intestinal Caco-2 cells. The British Journal of Nutrition 100, 542–551.
34. Babior, B.M., 2000. Phagocytes and oxidative stress. The American Journal of Medicine 109, 33-44
35. Preservative Effect of Pomegranate Peel Extract on the Keeping Quality of Cream Based Fat Spread Rashmi Bhardwaj1*, B. V. Balasubramanyam, Diwakar Mishra and Ashok Papasani, Dairy Technology Section, ICAR- NDRI (SRS), Bengaluru-560030, ISSN: 2320 – 7051 Int. J. Pure App. Biosci. 5 (6): 323-328 (2017)
36. Utilization of Pomegranate Peels for Improving Quality Attributes of Refrigerated Beef Burger Abdel Fattah A.A., Nadia R. Abdel-Rahman, M. M. Abd El-Razik* and Hafssa, B. El-Nashi Food Science Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt., Current Science International ISSN: 2077-4435 Volume : 05 | Issue : 04 | Oct.- Dec. |2016 Pages: 427- 441
37. Amy B. Howell and doris H. D'Souza. The Pomegranate: effect on bacteria and viruses that influence human health. volume2013, article ID 606212.

Advancement in Nanotherapeutics for Alzheimer's disease

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ABSTRACT

Background: CNS issues have reached globally. While quite a few studies is being encouraged, there aren't many capsules within side the marketplace to fight the severity of Alzheimer`s illnesses. Most of the medicine used for the control of those illnesses have terrible solubility, low hydrophilcity and cannot attain the goal thanks to the Blood-Brain-Barrier (BBB). Due to those problems, technological know-how related to nanotechnology is now getting used to formulate drug transport structures to permeate thru the barrier and supply the drug on the goal.

Methods: This mini evaluation analyses the research of numerous scientists and studies corporations on this area and discusses the importance of nanoparticles for drug transport throughout the BBB. Formulations which have tested excessive cappotential for penetration thru the bloodbrain- barrier encompass Solid Lipid Nanoparticles (SLNs), Polymeric Nanoparticles (PNs), Liposomes and Dendrimers.

Results: The control of those situations at a worldwide degree calls for efforts from a couple of studies disciplines in conjunction with a diagnostic platform, components improvement, and drug transport techniques

Conclusion: This mini evaluation discusses the destiny of a number of the maximum powerful nano-sized formulations for the remedy of Alzheimer`s disease.This mini evaluation makes a speciality of the nano-particulate structures and the destiny of nanomedicines for handing over capsules through the barrier for Alzheimer`s Disease (AD).

Keywords: Blood-brain-barrier (BBB), Alzheimer`s disease (AD), strong lipid nanoparticles (SLNs), polymeric nanoparticles (PNs), dendrimers, liposomes

Introduction:

Alzheimer's unwellness (AD) is that the most typical type of insanity worldwide, calculable to represent up to 50–80% of cases [1]. without doubt, AD can critically burden economies and health systems since cases square measure expected to succeed in 131 million by 2050 [2]. AD is usually

diagnosed symptomatically through the incidence of serious cognitive state, international psychological feature decline, and therefore the impairment of standard of living activities. Later within the course of the unwellness, the breakdown of physical functions, like walking, swallowing, and general movement, ultimately results in death [3]. insanity was the fifth-leading explanation for death in 2016.Currently there square measure solely four authority approved treatments for AD, and these square measure coupled in the main to the 2 molecular pathways involving the buildup the buildup amide and neurofibrillary tangles (NFT) of p-tau supermolecule [4-6]. However, none of those medication stops unwellness progression or cures AD, highlight the necessity for extra treatment approaches. The invention of novel biomarkers is hoped to deliver earlier AD identification and will conjointly support the identification of further molecular targets, probably resulting in new treatments.[7]

Identifying the pathophysiological processes concerned in AD and therefore the best biomarkers to find them is vital for the event of novel cures. Additionally, with efficiency and specifically delivering the diagnostic and therapeutic molecules to the sites of interest in these processes is vital. Nanoparticles (NPs) have enabled nice strides towards the delivery, treatment, and medicine of diseases, in the main because of their varied chemical characteristics and their propensity for chemical modification to modulate and refine needed properties [11, 12]. NPs' core constituents comprise a large style of materials, like lipids, polymers, and metals, which may encapsulate molecules with completely different chemical natures. Additionally, these carriers promote the protection and delivery of bioactive molecules, which may scale back their potential toxicity and, in turn, enhance their solubility, stability, biodistribution, and materia medica. Molecules encapsulated in NPs vary from little molecules, peptides, and proteins to genetic material [13].

AD was initial delineate in 1906 [15]. For an extended time then, firm identification was created once signs of cognitive state and psychological feature decline were already considerably advanced. Nowadays, several observations indicate that the pathophysiological alterations of AD within the brain begin decades before the onset of clinical symptoms of insanity.

Blood Brain Barrier

BBB structure isn't the main target of this review, thus some essential key points concerning this subject area unit given and also the reader is recommended to eventually leaf through different papers that higher gift its structure, physiological, immunologic and pathological characteristics and BBB overcoming methods.[16-18] In outline, BBB consists by brain microvascular epithelium cells, pericytes, astrocytes, tight junctions, neurons, and basal membrane that make never-ending barrier whose functions area unit the protection of the brain and also the strict management of the passage of solutes. Molecules which will overcome the BBB through passive diffusion ought to be liposoluble and customarily not charged at physiological pH scale.

Nanocarriers as diagnostic tools in AD

Polymeric based Nanoparticles

Nanoparticles (NPs) exposure can cause reduction in β-soluble proteins

e.g., functionalized with PEG and antibody, zinc loaded polymeric NPs, sitagliptin loaded NPs, curcumin loaded with chitosan and bovine serum albumin NPs etc.

Nanomicelles

Nanomicelles effectively mediate degradation of tau proteins at target sites

e,.g.; PEG ceramide nanomicelles

Dendrimers

These are synthetic polymers with a structure of repeatedly branching chains, to increase drug solubility and B.A for better permeation through B.B.B

E.g., Dendrimers with ethylenediamine core, nanocomposites of polyamidoamine dendrimers

Nanogels

They hold inhibit formation of amyloid proteins

e.g., Deferoxamine in the form of nanogel

Solid lipid Nanoparticles

Excellent carriers for bisabolol in A.D. brain

e.g., Solid lipid NPs loaded with curcumin

Liposomes

They deliver substantial conc. of genes to target tissues

PEG coated liposomes, glutathione-PEGylated liposomes, curcumin loaded liposomes etc

Neosomes

They act against amyloid aggregation

E.g. Niosomes loaded with artemisia-absinthium

Nano-emulsions

They bypass the B.B.B as they are formed using homogenization and ultrasonication

E.g. Nanoemulsion from naringerin

Cubosomes

Cubosomes are lipid based NPs and amylolipid nanovesicles which are self assembles lipid-modified starch hybrid system that have been proved to be promising carriers for delivery to affected parts of brain.

Others

Selenium NPs, Cerium NPs, Gold NPs, Iron NPs, Protein coated NPs, Antibody- decorated NPs, and other metallic NPs have recently gained the limelight in the fiels of nanomedicine for treating A.D

Conclusion:

AD is the fifth-leading cause of death worldwide, accounting for 2.4 million deaths yearly.The expected increase in the number of cases of dementia in the next few decades is even more important, given that there are currently no effective disease-modifying treatments for AD. Progress in AD management depends on innovation, the assessment of new candidates, and the implementation of new trial approaches. In this regard, recent advances on nano-medicine-drug development and novel diagnostic biomarkers could represent a promising alternative in the management of AD, as well as other neurodegenerative diseases.

References:

1. Nichols E, Szoeke CEI, Vollset SE, Abbasi N, Abd-Allah F, Abdela J, et al. Global, regional, and national burden of Alzheimer ' s disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019;18:88–106.
2. Prince PM, Prince M, Prince M, Karagiannidou M, Prince M, Karagiannidou M, et al. World Alzheimer Report 2016 Improving healthcare for people living with dementia. 2016.

3. Cummings JL, Isaacson RS, Schmitt FA, Velting DM. A practical algorithm for managing Alzheimer' s disease: what, when, and why ? Ann Clin Transl Neurol. 2015;2(3):307–23.

4. Anand R, Dip K, Ali A. Therapeutics of Alzheimer' s disease : past, present and future. Neuropharmacology. 2014;76:27–50.

5. Mendez MF, Program BN, Greater VA, Angeles L. Early-onset Alzheimer's disease. Neurol Clin. 2017;35(2):263–81.

6. Hang T, Seelaar H, Melhem S, Rozemuller AJM, Van Swieten JC. Genetic screening in early-onset Alzheimer' s disease identified three novel presenilin mutations. Neurobiol Aging. 2020;86:201.e9-201.e14.

7. Ettcheto M, Cano A, Busquets O, Manzine P, Sánchez-López E, Castro-Torres R, et al. A metabolic perspective of late onset Alzheimer ' s disease. Pharmacol Res. 2019;145:104255.

8. Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Maricarmen P, Sepehrband F. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat Med. 2019;25:270–6.

9. Nutma E, Amor S, Peferoen LAN. Astrocyte and oligodendrocyte cross-talk in the central nervous system. Cells. 2020;9:600.

10. Cummings J, Zhong K, Lee G, Ritter A, Sabbagh M. Alzheimer' s disease drug development pipeline: 2020. Alzheimer's Dement. 2020;6:e12050.

11. Mulvihill JJ, Cunnane EM, Ross AM, Duskey JT, Tosi G, Grabrucker AM. Drug delivery across the blood–brain barrier: recent advances in the use of nanocarriers. Nanomedicine (London). 2020;5(2):205–14.

12. Duskey J, Belletti D, Pederzoli F, Vandelli M, Forni F, Ruozi B, et al. Current strategies for the delivery of therapeutic proteins and enzymes to treat brain disorders. Int Rev Neurobiol. 2017;137:1–28.

13. Furtado D, Bjornmalm M, Ayton S, Bush AI, Kempe K, Caruso F. Overcoming the blood-brain barrier: the role of nanomaterials in treating Neurological Diseases. Adv Mater. 2018;30(46):e1801362.

14. Harilal S, Jose J, Parambi DGT, et al. Advancements in nanotherapeutics for Alzheimer's disease: current perspectives. J Pharm Pharmacol. 2019;71(9):1370–1383.

15. Kevadiya BD, Ottemann BM, Thomas MB, et al. Neurotheranostics as personalized medicines. Adv Drug Deliv Rev. 2019;148:252–289.

16. Ross C, Taylor M, Fullwood N, Allsop D. Liposome delivery systems for the treatment of Alzheimer's disease. Int J Nanomedicine. 2018;13:8507–8522.

17. Wong KH, Riaz MK, Xie Y, et al. Review of current strategies for delivering Alzheimer's Disease drugs across the blood-brain barrier. Int J Mol Sci. 2019;20:2.

18. Dong X. Current Strategies for Brain Drug Delivery. Theranostics. 2018;8(6):1481–1493.

ENVIRONMENTAL BIOTECHNOLOGY IN POLLUTION DETECTION AND MONITORING

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Abstract:

Environmental biotechnology is increasing in popularity which leads the global requirement to feed the world's growing population while also maintaining clean land, air, and water. Plant and microbial biology have seen significant technological advancements. Microorganisms are basically used for their catalytic variety and ease of genetic engineering, whereas plants can be more easily altered for resistances that increase yield or develop new products. This chapter depicts the application of environmental biotechnology and approach in pollution detection and monitoring. It is essential approach to the new researchers to integrate the biotechnology in the various environmental concerns to combat related issues easily by new developments.

Keywords: Environmental biotechnology, microbial biology, microorganism, pollution detection, pollution monitoring

Introduction:

Biotechnology is defined as "the combination of natural sciences and engineering to apply organisms, cells, components thereof, and molecular equivalents for products and services." [22]. Biotechnology is a flexible field that has had a significant impact on a broader range of technologies based on the use of biological processes in manufacturing, agriculture, food production, pharmaceuticals, restoration of environment, and resource conservation [3, 7, 9, 10, 11]. This new wave of technological change has resulted in significant improvements in a variety of fields (drugs, vitamins, steroids, interferon, fermentation products used as food or drink, energy from waste and renewable resources, additionally genetic engineering applied to plants, animals, and humans), as it has the potential to open up whole new paths for the long-term manufacturing of existing and new products, as well as serendipity. [18, 5, 11].

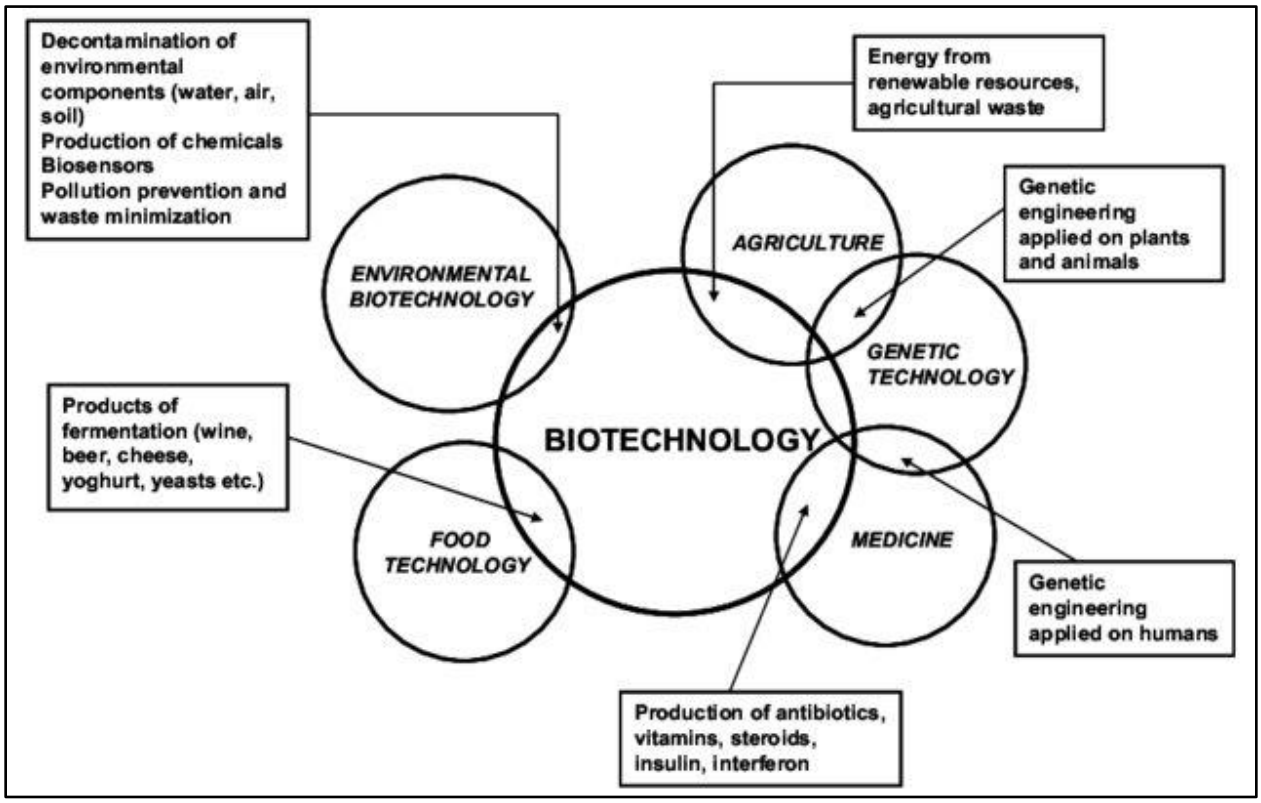


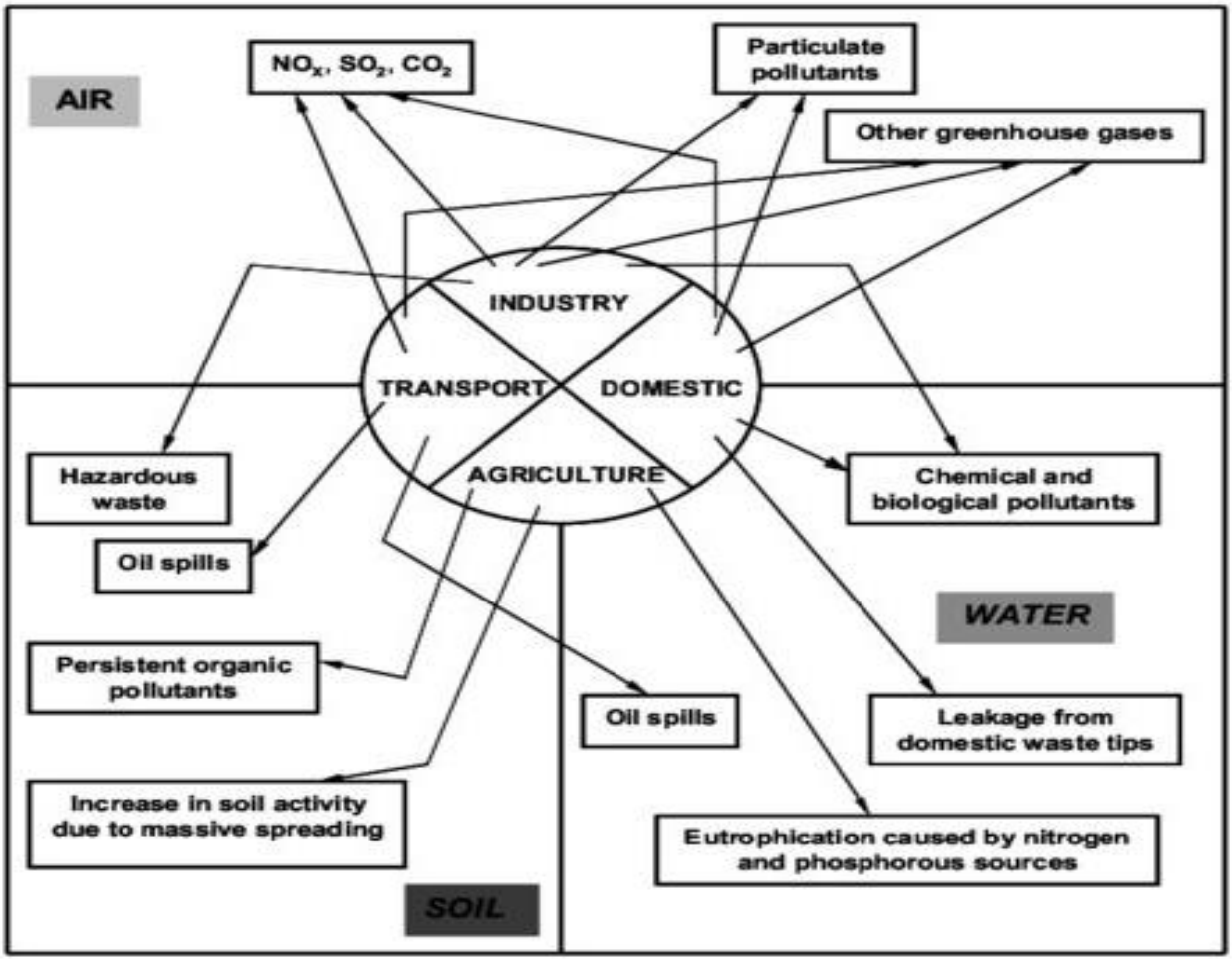
Figure 1: Application of Biotechnology in Anthropogenic Activities **Sources: [12]**

Furthermore, environmental concerns push the use of biotechnology not only for pollution treatment (water, air, and soil decontamination), but also for pollution prevention and waste minimization in the first place, as well as ecologically friendly chemical synthesis and bio monitoring.

Environmental monitoring is the process of assessing the quality of the environment by measuring a set of selected criteria on a regular basis. In general, two main approaches for the measurement and estimation the extent of pollution: physicochemical and biological [17, 19, 14, 15, 4]. In previous decades, environmental monitoring programmes mostly focused on measuring physical and chemical factors, with biological variables thrown in on occasion. The use of analytical equipment is required for physicochemical procedures, which have two drawbacks: cost (because to the complexity of the samples and the competence of the operators required to execute the analysis) and inadequate hazard and toxicological information [2, 13].

It has difficulties to monitor the environment in order to protect it. Because of the negative effects of toxic chemicals on natural ecosystems, there is a growing demand for early warning systems that can detect toxicants at very low concentrations [6].

Figure 2: Web of Environmental Pollution due to Anthropogenic Activities



Sources: [12]

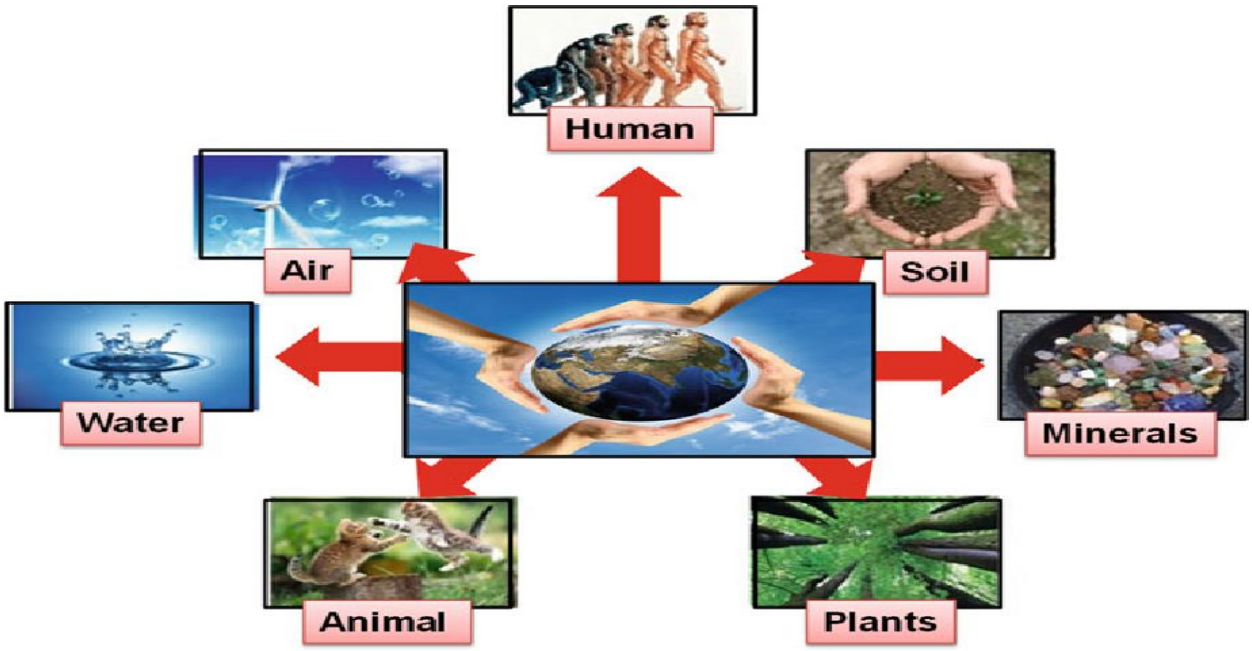
Typically, contamination monitoring entails measuring numerous chemicals in water, soil, sediment, and air on a regular and frequent basis during a set time period, such as a year. Environmental biotechnology combined with information revolutionized our ability to monitor and manage processes at the molecular level "in order to attain real-time information and computational analysis in complex environmental systems" [16].

Table1: Pros and Cons of Environmental Biotechnology

Advantages	Disadvantages
1. Environment Biotechnology is an answer to the world's growing requirement of food by providing more feasible techniques for farming.	1. Experiments on Animals harm them and even though if the tests are done to protect the species in the future they are still suffering in present.
2. It decreases the environmental consequences like greenhouse gases, etc. that are the results of agricultural and various other activities.	2. While experimenting there are always some risks that come handy with the results. Here, if we talk about nature and biodiversity; while performing any test, even though it is for good, there are a huge amount of risks predicted.
3. It benefits nature, for instance, Conservation Tillage is a technique developed by the Environmental biotechnologists that reduced the amount of carbon dioxide gas in the air to a great extent.	3. Environmental Biotechnology is often questioned if it may exploit the lives of forest tribal people.

Sources: [8]

Figure 3: Scope and Close Association of Environmental Biotechnology with Planet Earth



Bioindicators/biomarkers: Apart from chemical measurements in physical compartments, environmental monitoring programmes have recently incorporated the determination of contaminant levels in biota, as well as the assessment of various responses/parameters of biological/ecological systems. Biomonitoring can now employ temporal and geographical changes in specified biological systems/parameters to represent changes in environmental quality/conditions [20, 4, 19]. Some organisms or communities may respond to an environmental effect by changing a measurable biological function and/or chemical composition in this context. In this method, significant environmental change can be inferred, and the responses are referred to be bio indicators/bio-markers [21, 17, 20, 4].

As a result, biomarkers are employed in biomonitoring programmes to provide biological information, such as the impact of contaminants on live organisms. Exposure, effect, and susceptibility are the three basic types of indications that can be collected. The following biomarkers have the potential to be used in biomonitoring: - molecular (gene expression, DNA integrity) biological chemistry (enzymatic, specific proteins or indicator compounds) cytopathological histopathology (cytological, histopathological) - physiological and behavioural aspects Unfortunately, the use of biomarkers in the field is constrained by a variety of factors (for example, the availability of living material), which might limit data collection and prevent the use of multivariate methods during statistical analysis. They should also have the following

characteristics: be sensitive (to act as an early warning), specific (either to a single compound or a class of compounds), broad application, easy to use, reliable and robust, good for quality control, able to be easily taught to personnel, and provide the data and information required [1].

Conclusion:

Environmental Biotechnology is a vast topic of study that has a significant impact on the country's development. It aids in the cleanup of the environment and the development of remedies to prevent additional contamination. Environmental Biotechnology has seen a number of advancements, and future advancements will undoubtedly increase its scope. To manage scientific duties, several of the new technologies currently use genetically modified biological forms. Bioinformatics assembles data that is then used to aid bioremediation by providing data for microbial knowledge. Nanotechnology-based cleanup has also proven to be beneficial. Biotechnology has the potential to make even more significant contributions to the security and remediation of natural contaminants.

Acknowledgement:

I am thankful to my PhD Guide Dr. Swapnil Rai and Head of Institution (HoI) Dr. Kuldip Dwivedi for their continuous support and guide during my whole research work and difficulties faced during new emerging technologies in the field of research.

References:

[1] Beliaeff B, Burgeot T (2002) Integrated biomarker response: a useful tool for ecological risk assessment. Environmental Toxicology and Chemistry 21, 1316-1322

[2] Cannons AC, Harwood VJ (2004) Sensor Technology for Water Quality Monitoring: Fiber-Optic Biosensor (WERF Report), IWA Publishing, London, 72 pp

[3] Chisti Y, Moo-Young M (1999) Fermentation technology, bioprocessing, scaleup and manufacture. In: Moses V, Cape RE, Springham DG (Eds) Biotechnology: The Science and the Business (2nd Edn), Harwood Academic Publishers, New York, pp 177-222

[4] Conti ME (2007) Biological Monitoring; Theory and Applications, Bioindicators and Biomarkers for Environmental Quality and Human Exposure Assessment, WIT Press, Southampton, UK, 228 pp

[5] Das TK (2005) Toward Zero Discharge. Innovative Methodology and Technologies for Process Pollution Prevention, John Wiley and Sons, Hoboken, New Jersey, 744 pp

[6] Durrieu C, Tran-Minh C, Chovelon JM, Barthet L, Chouteau C, Védrine C (2006) Algal biosensors for aquatic ecosystems monitoring. The European Physical Journal - Applied Physics 36, 205-209

[7] EC (2002) Life Sciences and Biotechnology - A Strategy for Europe, Communication from the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions. Online at: http://ec.europa.eu/biotechnology/pdf/com2002-27_en.pdf

[8] Environmental
Biotechnology. 2020. Advantages And Disadvantages.(https://theworldbehindenvirobiotech.weebly.com/advantages-and-disadvantages.html)

[9] Evans GM, Furlong JC (2003) Environmental Biotechnology. Theory and Application, John Wiley and Sons, Chichester, 300 pp

[10] Gavrilescu M (2004) Cleaner production as a tool for sustainable development. Environmental Engineering and Management Journal 3, 45-70

[11] Gavrilescu M, Chisti Y (2005) Biotechnology-a sustainable alternative for chemical industry. Biotechnology Advances 23, 471-499

[12] Gavrilescu, M. (2010). Environmental biotechnology: achievements, opportunities and challenges. *Dynamic biochemistry, process biotechnology and molecular biology*, *4*(1), 1-36.

[13] Gu MB, Mitchell RJ, Kim BC (2004) Whole-cell-based biosensors for environmetal biomonitoring and application. In: Zhong JJ (Ed) Biomanufacturing, Scheper T (Series Ed) Advances in Biochemical Engineering/Biotechnology, Springer-Verlag Berlin Heidelberg New York, pp 269-305

[14] Hagger JA, Jones MB, Leonard DRP,Owen R, Galloway TS (2006) Biomarkers and integrated environmental risk assessment: Are there more questions than answers? Integrated Environmental Assessment and Management 2, 312-329

[15] Hart JK, Martínez K (2006) Environmental Sensor Networks: A revolution in the earth system science? Earth-Science Reviews 78, 177-191

[16] Hashim MA, Uijang Z (2004) Environmental biotechnology: its relevance and prospects for developing countries. In: Vjang Z, Menze M (Eds) Environmental Biotechnology, IWA Publishing, pp 7-12

[17] Jamil K (2001) Bioindicators and Biomarkers of Environmental Pollution and Risk Assessment, Science Publishers, New Hampshire, USA, 204 pp

[18] Johnston DJ (2003) Biotechnology: the next wave of innovation technologies for sustainable development. In: Seralgedin I, Persley GJ (Eds) Biotechnology and Sustainable Development: Voices of the South and North, CABI Publishing, pp 67-74

[19] Lam PKS (2009) Use of biomarkers in environmental monitoring. Ocean and Coastal Management 52, 348-354

[20] Markert BA, Breure AM, Zechmeister HG (2003) Definitions, strategies and principles for bioindication/biomonitoring of the environment. In: Markert BA, Breure AM, Zechmeister HG (Eds) Bioindicators and Biomonitors, Elsevier Science, Oxford, pp 3-39

[21] NRC (1987) Biological markers in environmental health research (National Research Council. Committee on biological markers). Environmental Health Perspectives 74, 3-9

[22] van Beuzekom B, Arundel A (2006) OECD Biotechnology Statistics – 2006. OECD, Available on-line at: http://www.oecd.org/dataoecd/51/59/36760212.pdf

Phytochemistry and Ethnomedicinal Leafy Plants Used by Tribal People of District Kondagaon Chhattisgarh

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Abstract

All over the world, raw parts of leafy plants and their extracts are used in medicinal products this also. It is estimated that 25% of modern medicines are derived. Most of which are from medicinal leafy. There are over 10 leafy plant species like *Brassica compestris, Amaranthus spinosus, Bauhinia purpurea* etc. and their uses are body weakness, menstrual disorder, leucorrhoea, internal pains, fever, urinary troubles and diarrhea, aches and pains, fever, dysentery, tumors etc. these leafy vegetable plants from the backbone of medicines. Now, my phytochemical study 10 wild leafy vegetable plants that were found. The studied medicinal plants are the source of the primary and secondary metabolites i.e., alkaloids, terpenoids, saponins, phenols, flavonoids, tannins Carbohydrates, Proteins and glycosides. In my study which is more present in 8 phytonutrients out of 9 in plant *Ficus mollis* and absent in Saponins. So, in *Bauhinia purpurea* only 4 phytonutrients is present that is carbohydrates, tannins, flavonoids and proteins.

Keywords: Medicinal leafy plants, Extracts, *Brassica compestris*, Phytochemical, Primary and Secondary metabolites, *Ficus mollis*, *Bauhinia purpurea*

Introduction

Green verdant vegetables are famous all over the planet. Heavy metal defilement of vegetables can't be undervalued as these staples are significant parts of human eating regimen. Ethnobotany is the logical investigation of the connections that exist between neighborhood ancestral individuals and green leafy plants. The word ethnobotany in a real sense implies the investigation of herbal science of the primitive human race. This term was first applied by Harshberger in 1895- to investigation of plants utilized by primitives and aboriginal people. The term ethnobotany has been differently characterized and deciphered by ensuing workers. (Jones, 1941) defined it as a study of the interrelations of primitive man and plants. After a decade, Jones revised his concept and treated ethnobotany as a unit of an ecological study specializing in the interaction of man and plants. As indicated by (Harshberger, 1895), the term ethnobotany was presented for the utilization

of plants by the aboriginal people. As indicated by (Harshberger, 1885), the expression "Aboriginal" Botany was presented for the investigation of all types of vegetation which natives utilized for wares like medication, food, materials and ornamentals. As per (Vertek Gadgil, 1986) ethnobotany is a part of monetary plant science a segment of which manages the job of plants in the life and culture of aboriginates and ancestral individuals. As indicated by (Mudgal and Jain, 1984), ethnobotany manages studies among the ancestral's kin of recording their exceptional information about plant riches and for search of new assets of home grown, drugs, eatable plants and different parts of plants. As indicated by (Jain, 1980), ethnobotany manages the immediate connections of plants with man. Ethnobotanical study is the perplexing connection among plants and culture. The focal point of ethnobotany is on how plants have been utilized overseen and seen by the human social orders and incorporate plants utilized for food, medicinal, cosmetics, dying, textiles for building, tool, currency, clothing and social life.

The expression "phyto" began from a Greek word significance plant. Phytonutrient or certain organic components of plant and these components are through to promote human health (Lenta *et al.*, 2007). Phytochemicals are present in apart of leafy plants which are make use of vital components of both human and animal diets. These include fruits, seeds, herbs, roots, leaves and vegetables (Okwu, 2005). Significant piece of the verdant plants are eaten or utilized for their rich phytochemical fixings, which give both preparatory and healer characters to purchasers against illnesses a large portion of which have had an age long entity. Many more researchers worked to check different verdant plant compound fixings in India and Abroad (Kapoor et al.,1969, Edeoga et al., 2005 and Prohp and Onoagbe, 2012). Phytochemicals are essentially isolated into two gatherings i.e., primary constituents it includes of common carbohydrates, amino acid, proteins and chlorophyll. Secondary constituents it includes of alkaloids, Terpenoids, saponins, phenols, flavonoids, tannins, glycosides and many more.

As indicated by my field study, those wild consumable verdant plants are accessible in the area of Kondagaon, Chhattisgarh. The local tribal people of the area have traditionally used and consumed the raw and boiled of those wild leafy vegetables, but they do not cultivate it. The purpose of this research is to study the nutritional composition ethnobotany of wild leafy vegetables which indicates ancient wisdom diet and medicinal uses. However many analysts detailed and archived the nutritional composition and medical advantage capability of wild food plants, including wild

leafy vegetables of Kondagaon. As identifying, describing, cataloging and explaining the medicinal plants of "Kondagaon district of Chhattisgarh".

Materials and Methods:-

Study Sites: The study was conducted of districts Kondagaon Chhattisgarh. Kondagaon is newly formed District of Chhattisgarh state. It is situated in south eastern part of the State in Central India. It falls in survey of India, between 19^0 11' to 20^0 13' North latitude and 81^0 17' to 82^0 04' East longitudes. Kondagaon District has an area of 7768.907 square kilometers. It has a population of 5,78,326, Out of the total population, more than 70% are tribal and comprised by Gond, Maria, Muria, Dhruva, Bhatra, Halba etc.

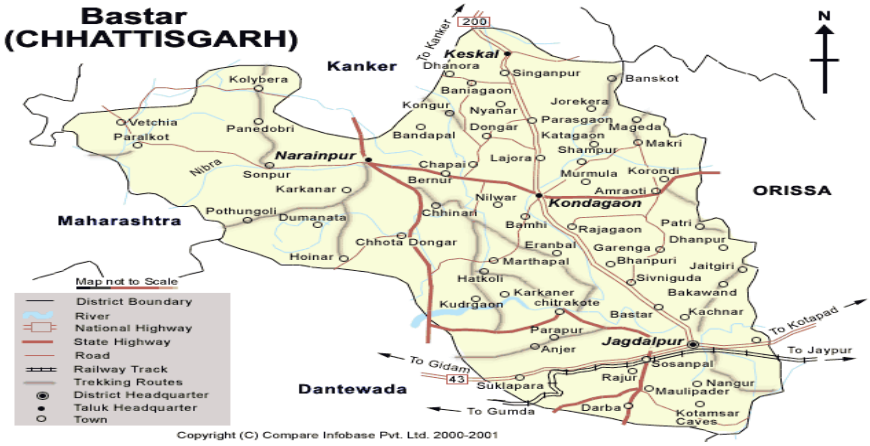

Fig.1: Map of Bastar (Chhattisgarh)

Climatic condition: Devarkar and Joshi (2011), was study on three main climatic seasons summer, winter and rainy. The colder time of year season starts of November and finishes center of February. The late spring season begins from center of February to May. In this way, rainy season begins from June to October. In this area climate is tropical and sub humid with annual air temperature of 27.0°C and annual rainfall of 1534 mm. The temperature system is isohyperthermic while dampness systems are udic and ustic. The dirts of Kondagaon locale are red sandy, red and earthy colored sandy soil, red and black, skeletal and black. The annual soil temperature is 26.0°C and in summer soil temperature is 29.3°C.

Collection and identification of leafy plant materials:-

To collect the leafy plants from my site Bastar (Kondagaon). The entire plants were gathered from crude regions situated at Kondagaon region of Chhattisgarh.

Preparation of plant material for further experiment: - Initially, the verdant plant parts which were chosen for phytochemical screening were totally washed with faucet water. Now, collected plant materials (leaf) were kept for shade dry for some days. After that the powder type of leaf was gotten which was further dipped in distilled water for 24 hours and go further for extraction.

Soxhlet extraction: - Nikhal *et.al*. (2010), studied about soxhlet extraction is only required where the desired compounds have a limited solubility in a solvent, and the impurity is insoluble in that solvent.

Protocols for phytochemical screening analysis are as follows:- Phytochemical tests were done on the watery concentrate and on the powdered examples utilizing standard system to distinguish the constituents as portrayed by Sofowara (1993) and Harborne (1973).

1. **Detection of Carbohydrates (Benedict's Test):-** 2ml of plant extracts and add few drops of benedict's reagent. The solution is then heated in a boiling water bath for 3-5 minutes. Brick red coloured indicates the presence of carbohydrates.
2. **Detection of Tannins:-** 2ml of the plant extracts and add 1ml of 5% $FeCl_3$. Green-Black colour appears and then indicates the presence of tannins.
3. **Detection of Proteins (Xanthoproteic Test):-** 2ml of the extricated test were treated with few drops of Conc. Nitric corrosive. Yellow tone demonstrates the presence of proteins.
4. **Detection of Terpenoids:-** 5ml of plant isolates were managed 2ml of chloroform and 3ml of Conc. H2SO4 Reddish Brown tone demonstrates the presence of Terpenoids..
5. **Detection of Phenols (Ferric Chloride Test):-** 1ml of plant separates were dealt with 5-6 drops of ferric chloride. Then Bluish-Black colour appears they indicates the presence of Phenols.

Results and Discussions

In my survey site I found 10 edible leafy plants used by Tribal people. These leafy plants are being used for many diseases and various part of the plant viz root, stem, leaves, fruits, flowers, bark etc. Ethnobotanical information have been assembled on the customary employments of verdant plant species given underneath in table.

Table 1: Uses of Ethnomedicinal Leafy Plants

S.No.	Ethnomedicinal Uses	Local Name	Botanical Name	Family	Used plant Parts
1	Body weakness, menstrual disorder, leucorrhoea and internal pains	Sarso Bhaji	*Brassica compestris*	Brassicaceae	Leaves & Seeds
2	Fever, urinary troubles and diarrhea, excessive menstruation, vaginal discharges, nose bleeding and wounds.	Kanta Bhaji	*Amaranthus spinosus*	Amaranthaceae	Leaves
3	Piles, diabetes and obesity, skin diseases, asthma and intestinal worm infection, and in dysentery and diarrhea	Koliaari Bhaji	*Bauhinia purpurea*	Caesalpiniaceae	Leaves, Stem, Flower & Bark
4	Burns and skin erruptions, lu, fever, urinary diseases, enteritis	Awali Bhaji	*Oxalis corniculata*	Oxalidaceae	Leaves, Stem
5	Anti-inflammatory, analgesic, antiviral and chest pains.	Bodi Bhaji	*Cordia subcordata*	Boraginaceae	Leaves
6	Aches and pains, fever, tumors. Specially its leaves are used for piles, gonorrhea, cystitis and dysuria.	Safed chech Bhaji	*Chorchorus olitorius*	Tiliaceae	Leaves

7	Burns, corns, cough, cystitis, fistula, prostatitis, scurvy, spasms, tumors and warts	Aloo Bhaji	*Solanum tuberosum*	Solanaceae	Leaves & Tubers
8	Cuts and wounds, blood purification and to maintain healthy body	Gasti Bhaji	*Ficus mollis*	Moraceae	Leaves
9	Leaves are used to treat for indigestion	Chanti Bhaji	*Polygonum plebeium*	Polygonaceae	Leaves, seeds, roots
10	Snakebite and also to control sugar, diuretic, refrigerant and depurative	Chiyur Bhaji	*Pilularia globulifera*	Marsileaceae	Leaves

Phytochemical Table: The presence of these constituents in 10 wild leafy vegetable plants that were found in my survey site during my research are shown in table. The **'+'** sign denotes the presence of that particular constituent in that particular leafy plant and the **'-'** sign denotes the absence of that particular constituent.

Table 2: Phytochemical Study of Wild Edible Leafy Vegetable Plants

S. No.	**Name of Plants**	**Carbohydrates**	**Saponins**	**Tannins**	**Flavonoids**	**Alkaloids**	**Proteins**	**Terpenoids**	**Phenols**	**Glycosides**
1	*Amaranthus spinosus*	+	+	+	-	-	+	-	+	-
2	*Bauhinia purpurea*	+	-	+	+	-	+	-	-	-

3	*Brassica compestris*	+	+	+	-	-	+	-	+	-
4	*Chorchorus olitorius*	-	-	+	-	+	+	+	+	+
5	*Cordia subcordata*	+	-	+	+	+	+	+	+	-
6	*Ficus mollis*	+	-	+	+	+	+	+	+	+
7	*Oxalis corniculata*	-	+	+	-	+	+	+	+	+
8	*Pilularia globulifera*	-	-	+	+	+	+	+	+	+
9	*Polygonum plebeium*	-	+	+	-	-	+	+	+	+
10	*Solanum tuberosum*	+	-	+	+	+	+	+	+	-

Conclusion

Kondagaon area is blessed with rich floristic variety with various ethnomedicinal plants of economic importance. Now a days it has been realized that the ethnomedicinal studies of different tribal areas were going to play a vital role for future. These leafy vegetable plants from the backbone of medicines. Phytochemical studies shows variety of nutrients present in plants and quantitative analysis shows the amount of those nutrients. Generally speaking this study reasons that the information about these significant advantages about plants ought to be investigated more

and should be shared more so that the pharmaceutical and other industries also should use all these data at its fullest utility. These study will help the mankind to evolve and mould the future of the human being whether it comes to medical science or any other industry.

Acknowledgement

One of the authors Himani Sandey is very thankful to my supervisor. I would like to express my deep and sincere gratitude to my Supervisor Dr. Lata Sharma, Associate Professor Department of Botany Dr. C. V. Raman University, Kota, Bilaspur, Chhattisgarh for her patient and constant encouraging guidance during the progress of my work.

References

- Devarkar V.D. and Joshi M.P. (2011). Phytosociological studies of Bodhghat area Dist. Dantewada with reference to importance value index (IVI). Bionano Frontier.
- Edeoga HO, Okwu DE and Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology 4 (7), 685-688.
- Gadgil, M. and Mches, V.M.- Homji 1986**,** *Localities of great significance to conservation of India's biological diversity* : Proc. Ind. Acad. of Sci. Annual plant sci. suppl. pp 165-180.
- Harborne J.B., (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman A. & Hall. London P 279.
- Harshberger, J.W. (1895), Some new ideas; the plants cultivated by aboriginal people and used in primitive commerce. The Evening Telegraph (Daily), Philadelphia, volume 62, pp: 2.
- Jain, S. K. and Borthakru, S. K. (1980). Ethnobotany of the mikirs of India. Econ. Bot. 34 : 264-272.
- Jones, L. L. (1941). Osmotic regulation in several crabs of the pacific coasts of North America. Journal of cellular and comparative physiology. 18 (1) :79-92.
- Kapoor, L.D., Singha, A., Kapoor, S.L., & Srivastava, S.N., (1969), Survey of Indian plants for saponins, alkaloids and flavonoids- 1 Lioydia. 32 : 297- 304 pp.
- Lenta, B.N., Noungoue, D.T., Devkota, K.P., Fokou, P.A., Ngouela, S., Tsamo, E. & Sewald, N. (2007). Acta Cryst. E63, o1282-o1284.

- Nikhal SB, Dambe PA, Ghongade DB, Goupale DC (2010). Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhlation. International Journal of Pharmaceutical Sciences 2 (1): 30-32.
- Okwu, D.E. (2005). Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. International Journal of Molecular Medicine and Advance Sciences,1(4): 375-381.
- Prohp T.P., and Onoagbe I.O., (2012). Determination of phytochemical composition of the stem bark of *Triplochiton scleroxylon* k. Schum. (sterculiaceae). Int. J. Appl. Biol. Pharm. Technol., 3(2): 68-75.
- Sofowora A. (1993). Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York. Pp 97-145.

International Journal of Advanced Research in Biotechnology and Nanobiotechnology
Vol. 3, Issue I, July 2022 **ISSN: 2582-3310**

BIOACTIVITY OF PLANT POWDER AGAINST *CALLOSOBRUCHUS MACULATUS* L. (COLEOPTERA: CHRYSOMELIDAE)

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Background:

The preservation or safeguarding of grains and pulse products against insect pests attacks is predominant for food security. Plenty of researchers worked and working on this area to fetch some novel environmentally compatible stored product control agents, which is the need of the hour to replace synthetic pesticides. Control agents that are safe alternatives and can replace the toxic fumigants that are simple and convenient to use are required to ensure food security among people. The exploration for secured and eco-friendly pest control agents has led us in exploring biological substances from plants and even from animals for potential alternatives. Attention has been given to the possible use of plant products or dry powders as promising alternatives to synthetic insecticides in controlling insect pests of stored products.

Method and Result:

Callosobruchus maculatus is the most consequential pests in stored legumes in tropical countries. Therefore, applying plants or their products is the recent method being investigated for insect pest control. Therefore, the main objective of this study was to control the cowpea bruchids by powder of plants biologically.

In the present study, three plant species were assessed for their efficacy against the pulse beetle. The experiments were conducted on three plant powders compared with untreated control. Among the plant powders evaluated by mixing with the seeds about 2 % w/w, the results show *Muntingia calabura* 2% was considered significantly the best compared to other treatments and caused 100 % mortality three days after treatment, which was followed by *Ocimum tenuiflorum* (78.88 %) and *Cassia auriculata* (57.33%) At six days after treatment highest mortality was observed in *Ocimum tenuiflorum* (69.99 %) and the similar mortality was noticed at 7 DAT (Day After Treatment.

Conclusion:

Study the effect of three insecticidal plant powders against *C. maculatus* infesting the stored brown chickpea *Cicer arietinum*. The results disclosed the potency of various powders, and among them, *M. calabura* 2 per cent leaf powder caused 100.00 percent mortality to pulse beetle three days after treatment. Therefore, resource-poor farmers can use botanicals, namely, M. calabura powder, to control pulse beetle in stored brown chick pea. They may not afford to buy chemical pesticides due to the high cost. Furthermore, botanical pesticides to control pulse beetle is an appropriate strategy to avoid contamination of the ecosystem and other hazards, since the synthetic pesticides are used by most of the farmers and in agro industries.

KEYWORDS

Callosobruchus maculatus, Muntingia calabura, Ocimum tenuiflorum,Cassia auriculata, agro industries, pesticides.

Introduction:

Proteins are conventionally said to be bodybuilders as they help grow and develop. Vegans or vegetarians mostly rely on plant protein completely, and pulses fulfil this nutrient. Pulses can be a healthy diet choice like sprouts, and par boiled and completely boiled, etc. However, grains and pulses are usually susceptible to pest infestations during their field growth and storage. When prone to infestation, the pulses and grains lose their nutritional value. The cowpea weevil *Callasobruchus maculatus* is the most common bruchid that infests the cowpea in a very ravenous manner [1]. Several studies report that *C. maculatus* is amid of the crucial insect pests attacking chickpea [2, 3, 4]. Previous works stipulated that *C. maculatus* was a major pest and greater threat of various food legumes making the grains inapposite for human consumption and trading [5]. The tiny grubs creep into the pulses and grains, causing serious damage as it is a polyphagous insect or pests as it has many host seed cowpea, mung beans, adzuki bean and black gram to complete its life cycle [6]. The present investigation analysis the pesticidal activity of three plants of different families and different parts. Plants chosen and the segments implimented for the investigation were based on the medicinal use of plants as the plant powder is applied in the food industry. Many times to control pest infestation, people synchronize their thought process rapidly with synthetic pesticides and fumigation techniques which backlog many health disadvantages to human society.

Materials and methods:

Rearing of test Insect:

Adults of *Callosobruchus maculatus* were initially collected from my home as the chickpeas got penetrated with the pests and that was driven to the laboratory at the Department of Zoology. The pulse beetle, *Callosobruchus maculatus,* was reared on brown chickpea(Kala chickpea) seeds in glass jars covered with muslin cloth by following the method developed by Credland and Wright [7]. *C. maculatus* was maintained at ambient environment (28 ± 2 °C) and relative humidity (70 ± 5%) conditions.

Preparation of plant powders:

Three herbage of different families and parts were collected,washed and used for the analysis. The parts used and the name is listed in Table 1. Shade dried at room temperature for twenty-five to thirty days and powdered using an electric pulverizer or dry mixer grinder used into fine powder. The powder is again sieved by utilizing a sieve [8]. The crushed botanical powders were kept in air tight containers at room temperature and properly covered or closed tightly to prevent the loss of physical and chemical properties and used for experiments.

Effect of insecticidal activity of plant powders on the adult:

Twenty grams of Desi chickpea were taken in Petri dishes. The powder of various plant parts at the rate of 2: 100 (w/w) were added to Desi chickpea seeds and mixed thoroughly. Thirty newly emerged adults were released into each Petri plates and kept in the laboratory. The activity was replicated thrice. Mortality (lack of movement , response to repeated restless movement) was recorded at one-day intervals up to a week [8]. The percentage of mortality was determined by the following formula:

$$\text{Percentage mortality} = \frac{\text{Number of } C.maculatus \text{ dead}}{\text{Number of } C.maculatus \text{ introduced}} \times 100$$

Results and Discussion:

In the present study, three plant species were assessed for their bioactivity or insecticidal activity against pulse beetle. The experiments were conducted on three plant dry powders compared with untreated control. Out of the plant powders evaluated by mixing with the seeds @ 2 percent w/w, the results show *Muntingia calabura* was found to be significantly the best compared to other

treatments and caused 100 percent mortality in three days after treatment, which was followed by *Ocimum tenuiflorum* (78.88 %) and *Cassia auriculata* (57.33%) At six days after treatment highest mortality was seen *Ocimum tenuiflorum* (69.99 %) were noticed and the same style was detected at 7 Days After Treatment. Many studies reported that *M. calabura* extract possesses pesticidal activity. For example, Some studies proved that *M. calabura* fetches pesticidal activity in the larvae and pupae of *Plutella xyllostella* [9]. The study suggested that the various parts of the *M. calabura* are multipurpose medicinal plants that render treatment to different diseases [10]. *The M.calabura plant also possesses anti-diabetic activity, relieves pain, cold and headache, and has* antibacterial and cytotoxic activity [11]. *M. calabura* leaves possess antivectorial activity, and it helps in the control of filarial vector *Culex quinquefasciatus* [12]. The application of plant powder induced many changes in the organisms, like the bruchids immediately decamp from the substratum where the powder concentration was high. The bruchids cuddle each other, and slowly their movement becomes very slow. The GCMS analysis of *M. calabura, Ocimum tenuiflorum* and *Cassia auriculata* disclosed the presence of certain important compounds like Sorbitol or D-Glucitol, Phytol found in the *M. calabura* possesses the pesticidal activity and is supported by the [13, 14] *C. auriculata* flower bewitch the presence of Pyrazole, Xylitol, resorcinol, Arabinitol [15] which strongly manifest the pesticidal activity and the earlier studies [16]too underpin the analysis of the above compounds. In the same way, the flower of *Ocimum tenuiflorum* enthrals the presence of thymol, quinoa, Neoisolongifolene, naphthalene and the results [17,18,19,20]and These compounds exist in the dense agglomeration and aside from this many other phytocompounds found in the botanical act synergistically against the *C. maculatus*. The present study furnishes conspicuous bioactivity of the three plants powder of different parts.

Conclusion:

A study of the effect of three insecticidal plant powders against *C. maculatus* infesting the stored brown chickpea *Cicer arietinum* disclosed the potency of various powders among them, *M. calabura* 2 % leaf powder caused 100.00 percent mortality to pulse beetle three days after treatment. Therefore, resource-poor farmers can use botanicals. Viz. *M. calabura* powder in controlling pulse beetle is stored brown chickpea as they may not afford to buy chemical pesticides due to high cost. Furthermore, using plant-based pesticides to control pulse beetle is an appropriate strategy to avoid adulteration in the ecosystem and other hazards since farmers and agro-industries currently use chemical pesticides.

Table 1: The plant species assessed against *Callosobruchus maculatus*

S. No	Common name	Botanical name	Family	Parts used
1.	Sarkaraipazhamaram	*Muntingia calabura*	Muntingiacea	Leaves
2.	Holy basil	*Ocimum tenuiflorum*	Labiatae	Leaves
3.	Aavarai	*Cassia auriculata*	Caesalpinaceae	Flower

Table 2: Insecticidal activity of different plant part powder against the *Callosobruchus maculatus.*

S. No.		**Treatments**	**% Adult mortality**							Mean
			1 DAT	**2 DAT**	**3DAT**	**4 DAT**	**5 DAT**	**6 DAT**	**7 DAT**	
1.		*Muntingia calabura*	40.33	52.66	100	100	100	100	100	91.50
2.		*Ocimum tenuiflorum*	25.33	31.66	40.33	50.33	61.76	69.99	78.88	51.61
3.		*Cassia auriculata*	7.77	13.55	21.33	29.76	37.33	46.88	57.33	30.56
4.		Control	0.00	4.33	6.55	10.33	15.66	17.33	20.33	10.64


M. *calabura* Leaves *M. calabura* tree


C. auriculata flowers *O. tenuiflorum* leaves

References:

1. Emeasor, K.C., Emosairue, S.O. and Ogbuji, R.O. (2007). Preliminary evaluation of theefficacy of mixed powders of Piper guineense (Schum and Thonn) and Thevetiaperuviana (Persoon) against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) Nigerian Journal of Entomology, 24: 114 – 118.
2. Jerraya A (2003) Principaux nuisibles des plantes cultivées et des denrées stockées en Afrique du nord: Leur biologie, leurs ennemis naturels, leurs dégâts et leur contrôle. Climat pub, Tunisie. 415pp
3. Haouel, H.S., Hedjal, C.M., Kellouche, A., Khouja, M.L., Boudabous, A., Mediouni, J.B.J., 2015. Management of three pests' population strains from Tunisia and Algeria using *Eucalyptus* essential oils. Ind. Crops Prod. 74, 551-556.
4. Mabrouk, Y., and Belhadj, O., 2012. Integrated Pest Management in Chickpea, New Perspectives in Plant Protection, Prof. Ali R. Bandani (Ed.), InTech, DOI: 10.5772/35487. Available from: http://www.intechopen.com.sci-hub.cc/books/newperspectives- in-plant-protection/integrated-pest-management-in-chickpea.
5. Melo, V. et al. 2010. Edible Insects Indigenous Food Worldwide. 15th World Congress of Food Science and Technology. Cape Town, South Africa.

6. Oke, O.A., Akintunde, E.M., 2013. Reduction of the nutritional values of cowpea infested with *Callosobruchus maculatus* (Coloeptera: Bruchidae). Int. J. Agri. Sci. 3, 30-36.
7. Credland PF, Wright AW. Factors affecting female fecundity in the cowpea seed beetle, Callosobruchus maculatus (Coleoptera: Bruchidae). J Stored Prod. Res 1989;25(3):125-139.
8. Govindan K, Nelson SJ. Effect of Piper nigrum seed powder and dust formulation on Callosobruchus maculatus (F.). Annals of Plt. Protect. Sci 2007;15(1):39- 42. 1.
9. Bandeira GN, Camara CAG, Moraes MM, BaRros R, Akhtar MS 2013, Insecticidal activity of *Muntingia calabura* extracts against larvae and pupae of diamondback, *Plutella xylostella* (Lepidoptera, Plutellidae)
10. Upadhye Mohini , Kuchekar Mohini, Pujari Rohini , Kadam Shailja, Gunjal Priya. Muntingia calabura: A comprehensive review. Journal of Pharmaceutical and Biological Sciences 2021; 9(2):81-87.
11. Adila SulfianS.,Iswan M.Yuso M., Isolation and identification of antibacterial and cytotoxic compounds from the leaves of *Mutingia calabura*. March2013,https://doi.org/10.1016/j.jep.2012.12.032
12. Aarthi. N., Abinaya S., Nithyasri G R N., Thanga Brindha T., Sneka P., Vijina C V. Phytochemical screening and Antivectoral activity of Muntingia calabura, Journal of Advanced Applied Scientific Research -ISSN: 2454-3225 Vol-3-5 July 2021: 43-48.
13. Gary J Puterka, William Farone, Tracy palmer, Anthony Barrington .Structure-function relationships affecting the insecticidal and miticidal activity of sugar esters, J Econ Entomol, 2003 Jun;96(3):636-44.
14. Giovanni Benelli, Roman pavela, Ettore drenaggi, Nicolas Desneux, Filippo Maggi- Phytol, (*E*)-nerolidol and spathulenol from *Stevia rebaudiana* leaf essential oil as effective and eco-friendly botanical insecticides against *Metopolophium dirhodum.* Induatrial Crops and Products, Volume 155,1 November 2020.
15. Donald E. Champagne , Murray B. Isman , Kelsey R. Downum , and G. H. Neil Towers, Insecticidal and growth-reducing activity of foliar extracts from Meliaceae, Chemoecology 4:165-173 (1993).
16. Edwin Burgess. R. and Christopher J. Geden. Larvicidal potential of the polyol sweeteners erythritol and xylitol in two filth fly species. , *Journal of Vector Ecology, July 2019,* Vol. 44,pg 11-17

17. Ming Xu, Ty Wagerle, Jeffrey K Long, George P Lahm, James D Barry, Rejane M, Smith Insecticidal quinoline and isoquinoline isoxazolines, Bioorg Med Chem Lett, 2014 Aug 15;24(16):4026-30.

18. Long Cheng, Peng Peng Cai, Rui-Rui Zhang, Liang Han, Cheng- Xia Tan, Jian-Quan Weng, Tian –Ming Xu, Xiang- Hai Liu. Synthesis and Insecticidal Activity of New Quinoline Derivatives Containing Perfluoropropanyl Moiety. Journal of heterocyclic chemistry, Volume 56, Issue 4, April 2019, pg 1312-1317.

19. Smriti Kala, Nisha Sogan , S. N. Naik , AmrishAgarwal & Jitendra Kumar.Impregnation of pectin-cedarwood essential oil nanocapsules onto mini cotton bag improves larvicidal performances. Scientific reports, Nature research, (2020) 10:14107.

20. Wang-Yu Zhao, Chao-Fu Yang, Li-Juan Han, Bo Zhou, Ai-Juan Li, Lu-Lu Liu, Zi- Wei Zhang, Zi-Yu Wang, Wen-Jun Wu & Ji-Wen Zhang, Design, synthesis, and insecticidal activities of propargyloxy-naphthalene sulfonamide derivatives, Journal of Asian Natural Products Research May 2021

International Journal of Advanced Research in Biotechnology and Nanobiotechnology
Vol. 3, Issue I, July 2022 **ISSN: 2582-3310**

A Comprehensive review on the ethno-botanical uses of ethno-medicinal wild plants bylocal community of Bilha block of Bilaspur district of Chhattisgarh

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ABSTRACT

This study in consequence plainly indicates the awareness of the local community with respect to the susceptibility of vital and rural systems in Bilha block of Bilaspur district of Chhattisgarh. In present study, it was aimed to conduct a special survey for ethno-botanical uses of ethno- medicinal wild plants **(**EWPs) of Bilha block to document all present ethno-medicinal 70 species belonging 38 families in this area. Present study depicts that traditional knowledge forms the basis for the treatment of various ailments among local communities. Villagers still use EWPs for treatment of common ailment used these wild plants *Dioscorea bulbifera L., Leucus aspera, Curcuma angustifolia, Acorus calamus, Oroxylum indicum, Euphorbia hirta, Salvia plabeja* and *Melia azadarachta* for various diseases like stomach ulcers, heart diseases, arthritis, constipation, dysentery, asthma, diabetes, skin diseases, obesity, piles, leprosy, cancer, menstrual disorders, tuberculosis, jaundice, respiratory disorders, rheumatism, malaria and skin diseases. Most of the wild plants preparations were single whole plants used and sometimes with other plant parts. Further preparations of medicines were free from side effects and easily available. Therefore there is an urgent need of comprehensive study and documentation of indigenous knowledge of local community about medicinal wild plant parts and usually used which were being passed verbally from old generation to younger generation.

Key words - Traditional knowledge, *Dioscorea bulbifera L.,* documentation and treatment of various ailments.

INTRODUCTION.

Ethnobotany is the study of relationship between plants and humans. Since ancient time, plants have been exploited for various purposes such as food and healthcare (medicine). Plants are vital part of traditional medicine. (Barman, D. *et al.* 2014). Throughout world, traditional medicine men called Biygas, Vaidyas and Ojhas employ plants singly or combined the other plants part

formulations to treat human as well as animal ailments. In this review, we present updated information on the ethno-botanical uses activities shown by EWPs by referring standard flora, journal articles and various search web engines (Panigrahi, G. and Murthi, S. K. 1989). Whole plant or certain parts of the plant like root, rhizome, corn, stem, barks, tender shoots, fruit, flower and seeds are used ethno-botanically in several countries for several purposes such as vegetable and in the treatment of diseases like asthma, diabetes, skin diseases, obesity, piles, leprosy, cancer, menstrual disorders, tuberculosis, jaundice, respiratory disorders, rheumatism, malaria and skin diseases etc.(Gireesha, J. and Raju, N.S. 2013).

The main goal of our study founded to propose improved traditional prescription developed from efficient plant extracts against various disorders (Chamila Kumari Pathirana 2020). EWPs used in traditional medicine can offer an alternative treatment for various diseases. During the field visits, data was also collected in groups on research region were also performed. A total of 70 species have been identified, divided into 38 families. Documentation of this important knowledge is urgently required. Certain plants are exclusively used by a few people for cured diseases e.g., stomach ulcers (06), heart diseases (03) arthritis (03), constipation (03), dysentery (05), asthma (05), diabetes (07), obesity (03), piles (04), leprosy (03), cancer (03), menstrual disorders (03), tuberculosis (03), jaundice (02), respiratory disorders (02), rheumatism (06), malaria (04) and skin diseases (05) etc. **(**Kar, A. and Borthakur, S.K. 2008).

The amended survey was used to collect information about EWPs in the research area from individual informants. Between March 2020 and September 2021, fieldwork was completed. There has been very little ethno-botanical research in this area (Bhatia, H. *et al.* 2015). The study's main goals were to (i) identify and explore plant species that are used locally for the treatment and prevention of various diseases, (ii) documentation of traditional medicinal plant recipes, including methods of preparation and dosage, (iii) final point had assess the plants' conservation status. For data collection, five plots selected in each site visits were undertaken in four different seasons. Each field visit lasted more than 15 days. On the basis of information provided by the local people and elders in the study region, a total of 120 informants were chosen.

Materials and Methods

Study site - Population of the village area is normally dependant on agricultural and naturally growing plants with associated forest products. The study area is located 18 km away from main

Bilaspur city of Chhattisgarh. Bilha is located in India 82.05 longitude and 21.96 latitude. Field survey was laid 1m.×1m. Quadrates lay in 5 plots at random on 15 village areas. There are 15 villages namely Dagauri, Dhamni, Bundela, Bartori, Amerikapa, Hardi, Jhal, Khapra khol, Parsada, Limtari, Khapri, Hirri, Guma, Amaldiha and Bilha have been selected for the investigation. Distribution pattern of **(**EWPs) are documented is given in table (Ahire, D.U. 2009).

Aims and Objectives :- The Present work has been undertaken pointing to make a systemic collecting of EWPs commonly consumed by local communities of Bilha block of the state of Chhattisgarh and to make natural analysis to create beneficial authenticity. The study will create awareness towards renewal, enrichment and fortification of precious EWPs resources. (Mishra, L. *et al*. 2014).

Result and Research Elaborations:-

All of the local communities were visited on a regular basis, and data was collected using the following procedures. First up all personal visits: personal visits were undertaken to the 05 village area throughout the study. Second were personal Interviews: personal interviews with competent individuals, such as village medicine men and women, traditional bone setters, and so on were performed. Information was gathered by asking questions in their native vernacular name during an interview session (Kasagana, V.N. and Karumuri, S.S. 2011). This Comprehensive literature review and different ethno-botanical surveys conducted in Bilha block showed that a series of EWPs are well-known and used in the treatment of various diseases in Bilha block are reported in the table no. 1.

Table No. 1- Distribution pattern of ethno-medicinal wild plants (EWPs) in Bilha block ofBilaspur district of Chhattisgarh

Name of disease	**Botanical name**	**Family**	**Vernacular name**	**Plant part used**
Stomach ulcers				
	Dioscorea bulbifera L.	Dioscoreaceae	Dang Kanda	Bulb
	Amorphophallus paeoniifolius N.	Araceae	JangaliSuran	Corm
	Cucumis calosus (Rottl.) Cogn.	Cucurbitaceae	Ban Kachro	Root
	Leucus aspera	Lemiaceae	Gummi bhaji	Leaves
	Physlis minima	Solanaceae	Chirpoti	Fruits
Heart diseases	*Curcuma aromatica*	Zingiberaceae	Janglihaldi	Tuberous roots
	Coleus rotundifolius	Lemiaceae	Kachru	Tuberous undergrou nd stem
	Acorus calamus	Acoraceae	Bach	Rhizome
Arthritis	*Oroxylum indicum*	Bignoniaceae	Sonpatta	Leaves
	Strobulanthus heiniyanus	Asparagaceae	Raksha	Bark
	Bauhinia variegate	Fabaceae	Kachnar	Leaves, flowers, fruits
Constipation	*Ipomia batata*	Convolvulaceae	Shakarkand	Tuberous roots
	Curcuma angustifolia	Zingiberaceae	tikhur	Tuberous roots
	Phoenix dactylifera	Araceae	Khajur	fruit
	Cassia tora	Caesalipiniaceae	charota	Leave seeds

Dysentery	*Dioscorea hispida Dennst.*	Dioscoreaceae	Baichandi	Bulb
	Aegle mormelos (L.)	Rutaceae	Bel	Leaves, fruits
	Madhuca indica	Sapotaceae	Mahua	Fruits
	Salvia plabeja	Lamiaceae	Memari	Seeds
	Brayophyllum pinnatum	Crassulaceae	Bhasampatti	Root, leaf
Asthma	*Stachys sericea*	Lemiaceae	Migina	Bulb
	Euphorbia hirta	Euphorbiaceae	Lal dudhi	Whole plants
	Zingiber officinale	Zingiberaceae	Adrak	Rhizome
	Calotropis procera	Asclepiadaceae	Aak	Whole plant
	Eclipta prostrata	Astaraceae	Bhengara	Whole plant

Diabetes	*Dioscorea pentaphylla L.*	Dioscoreaceae	Suvar Kanda	Bulb
	Catharanthus roseus	Apocynaceae	Sadabahar	Flower, leaves
	Tagetes erecta	Asteraceae	Marigold	Flower
	Achyranthes aspera	Amaranthaceae	Apmarg	Whole plant
	Melia azedarachta	Meliaceae	Bakain	fruits
	Syzygium cumini,	Myrtaceae	jamun	Leaves, Fruit, seed
	Gymnema sylvestre	Asclepiadaccac	Gudmar	Fruits
Piles	*Curcuma caesta*	Zingiberaceae	Kali haldi	Tuberous roots
	Amorphophallus campanulatus	Araceae	Jimikand	Corm, Tuber undergrou nd stem
	Sphaeranthus indicus Linn.	Astaraceae	Gorakhmundi	Whole plant
	Terminalia bellirica	Fabaceae	Bahera	Fruits
Malaria	*Andrographis paniculata*	Acanthacea	Bhui neem	Leaves
	Vitex negundo	Verbenaceae	Nirgundi	Leaves
	Centrather manthelminticus	Asteraceae	Vanjeera	Seeds
	Tinospora cordifolia, Willd.	Menispermaceae	Giloy	Stem
Leprosy	*Curcuma pseudomontna L.*	Zingiberaceae	Haldi	Rhizome
	Cynodon dactylon	Gramineae	Dubghas	Leaves
	Carissa carandas	Apocynaceae	karonda	Fruit
Cancer	*Urginea indica (Roxb.) Kunth*	Liliaceae	Jangalipyaz.	Bulb
	Mitragynaparvifolia	Lythraceae	Mehandi	Leaves, seed

	Manihot esculenta	Euphorbiacea	Panchpakhri	Tuberous roots
Menstrual disorders	*Aristolochia indica L*	Aristolochiaceae	Ishwarmul	Tuberous roots
	Foeniculum vulgare	Apiaceae	Souf	Seeds
	Morus nigra,	Moraceae	sahtut	Fruit
Obesity	*Pueraria tuberosa*	Fabaceae	Bidarikand	Root
	Lathyrus macrorrhizus	Fabaceae	Ful matar	Tuberous roots
	Buchananialanzan	Anacardiaceae	Char	Seed
Tuberculosis	*Fritillaria raylei*	Liliaceae	Kakoli	Bulb
	Cyperus rotundus L.	Cyperaceae	Nagarmotha	Tuberous root
	Asparagus racemosusWilld.	Liliaceae	Shatavar	Tuberous

				Roots
Jaundice	*CurculigoorchioidesGaertn.*	Hypoxidaceae	Kali Musli	Root
	Hygrophila auriculata	Acanthacea	Talmakhana	Seeds
Rheumatism	*Colchicum luteum*	Liliaceae	Hirantutia	Corm
	Chlorophytum borivilianum Santapau&Fernands	Liliaceae	Safed Musli	Tuberous root
	Listea monopetala	Liliaceae	Meda	Bark
	Solanum surattense	Euphorbiaceae	Bhatkataiya	fruit
	Asphodelus tenuifolius	Liliaceae	Pyazi	Bulb
	Commiphora wightii	Burseraccac	Guggul	Fruits
Respiratory disorders,	*Bambusa vulgaris*	Poaceae	Bans	Culm, shoots
	Ficus racemosa	Moraceae.	Gular	Fruit
Skin diseases	*Costus speciosus (J. Koeing)Sm*	Zingiberaceae	JangaliAadu	Rhizome
	Tamarindus indica	Caesalipiniaceae	Imli	Seeds
	Cassiya fistula L.	Caesalipiniaceae	Amaltas	Fruit, leaf
	Terminalia chebula	Combertaceae	Harra	Root
	Swertia chiraita	Gentianaccac	Chirayta	Fruits

Table 2: Diversity of EWPs Families

S.No.	**Family**	**Total No. of the EWPs**
1	Acanthacea	2
2	Acoraceae	1
3	Amaranthaceae	1
4	Anacardiaceae	1
5	Apiaceae	1

6	Apocynaceae	2
7	Araceae	3
8	Aristolochiaceae	1
9	Asclepiadaeae	2
10	Asparagaceae	1
11	Astaraceae	4
12	Bignoniaceae	1
13	Burseraccac	1
14	Caesalipiniaceae	3
15	Combertaceae	1
16	Convolvulaceae	1
17	Crassulaceae	1

18	Cucurbitaceae	1
19	Cyperaceae	1
20	Dioscoreaceae	3
21	Ebenaceae	1
22	Euphorbiacea	3
23	Fabaceae	4
24	Gentianaccac	1
25	Gramineae	1
26	Hypoxidaceae	1
27	Lamiaceae	4
28	Liliaceae	7
29	Lythraceae	1
30	Meliaceae	1
31	Moraceae	2
32	Myrtaceae	1
33	Poaceae	1
34	Rutaceae	1
35	Sapotaceae	1
36	Solanaceae	1
37	Verbenaceae	1
38	Zingiberaceae	6
	Total	**70**

In our study site result revealed that Bilha block, representing **70** EWPs and **38** families which has maximum (07) EWPs found in families Liliaceae and Zingiberaceae have (06) EWPs,Lamiaceae, Fabaceae, and Astaraceae have (04) EWPs, Araceae, Caesalipiniaceae, Dioscoreaceae, Euphorbiacea, (02) EWPs, Acanthacea, Apocynaceae, Asclepiadaeae, Moraceae and others family have only one EWPs found in Bilha block of the district Bilaspur C.G.given in table no.2.

This paper reviews the ethnomedicinal wild plants used for the treatment of stomach ulcers, heart diseases, arthritis, constipation, dysentery, asthma, diabetes, skin diseases, obesity, piles, leprosy,

cancer, menstrual disorders, tuberculosis, jaundice, respiratory disorders, rheumatism, malaria and skin diseases in Bilha block focusing on *Dioscorea bulbifera L*., *Fritillaria raylei*, *Asphodelus tenuifolius, Tamarindus indica, Swertia Chiraita, Chlorophytum borivilianum* etc. These plants were selected according to literature review and information collected from local communities traditional healers (Padal, S.B. *et al.* 2013).

CONCLUSION

Almost all species are commonly available in all sites of Kota block of Bilaspur C.G., but many people are not aware about their importance. Some species are facing threats due to deforestation, pollution, whether changes; global warming with various reasons and require immediate attention for their conservation (Verma, D. M. *et al.* 1993). From this study, it could be concluded that Bilha block of district Bilaspur possess a mixed vegetation. However,

occurrence with dominance was found to be shared by more than one species. Whole plants and different parts of plants are used in curing different diseases. Traditional information should be spread among other societies living in urban area and village area also (Verma, D. M. *et al.* 1985).

REFERENCES

- Ahire, D.U. (2009). Survey of some medicinal plants of Dindori Tahsil in Nasik District (MS). Env. and Cons. 15(2):2009; PP.(263-264).
- Barman, D., Nath, N. and Deka, K. (2014), Phenology of some medicinal plant species of Goalpara district, Assam (India). Scholars Academic Journal of Biosciences 2(2):81–84.
- Bhatia, H., Sharma, Pal Y., Manhas R.K. and Kumar K. (2015).Traditional phytoremedies for the treatment of menstrual disorders in district Udhampur, J&K, India. *J. Ethnopharmacol.* 2015;160:202–210.
- Chamila Kumari Pathirana, Terrence Madhujith, and Janakie Eeswara (2020). Bael (*Aegle marmelos* L. Corrêa), a Medicinal Tree with Immense Economic Potentials Advances in Agriculture / 2020 / Volume 2020 |ID 8814018 | https://doi.org/10.1155/2020/8814018.
- Gireesha, J. and Raju, N.S. (2013), Ethno-botanical study of medicinal plants in BR hills region of Western Ghats, Karnataka. Asian journal of Plant Science and Research 3(5):36–40.
- Kar, A. and Borthakur, S.K. (2008), Medicinal plants used against dysentery, diarrhea and Cholera by the tribes of erstwhile Kameng district of Arunachal Pradesh. Natural product

Radiance 7(2):176–181.

- Kasagana, V.N. and Karumuri, S.S. (2011), Conservation of medicinal plants (Past, Present and future trends). Journal of Pharmaceutical Science and research 2011; 3(8):1378– 1386.
- Mishra, L., Dixitand, Y and, Singh, M. (2014), Studies on ethnomedicinal plants of shekhawati region, Rajasthan, having hypoglycemic properties. Indian Journal of Fundamental and Applied Life Sciences 4(2):62–66.
- Padal, S.B., Chandrasekhar, P. and Satyavathi, K. (2013), Ethnomedicinal investigation of medicinal plants used by the tribes of pedabayalu mandalam, Visakhapatnam district, A.P., India. International Journal of computational engineering research 3(4):8–13.
- Panigrahi, G. and Murthi, S. K. (1989), Flora of Bilaspur: Vol. 1. Botanical Survey of India, Calcutta.
- Verma, D. M., Balakrishnan, N. P. and Dixit, R. D. (1993), Flora of MadhyaPradesh : Vol. I. Botanical Survey of India, Calcutta.
- Verma, D. M., Pant, P. C. and Hanif, M. I. (1985), Flora of Durg, Rajnandgaon and Raipur.Botanical Survey of India, Calcutta.

Study of Microbial Growth Kinetic Parameters of Cellulase Enzyme Production for Biofuel Application

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Abstract

Cellulase Enzyme is a class of biological catalyst which used to degrade polysaccharide i.e. Cellulose into simple sugar, thus it will help in breaking down the one of major components of plant cell walls i.e. https://en.wikipedia.org/wiki/Hemicellulose celluloses which is a major component of lignocellulosic biomass and its breakdown plays major role in bioethanol production. Cellulose can be hydrolyzed to glucose monomers using chemicals such as strong acid or biological catalysts cellulase. Cellulase enzymes can be produced from various microorganisms such as fungi, bacteria yeast, and some animals also. For our study, we consider ***Aspergillus Niger*** as the most potent and easily producible strain by using soil on malt extract agar and potato dextrose agar medium which confront the growth of enzyme in large quantity. By using a potent strain rate of growth of cellulase enzyme can be improved in lignocellulossic biomass. Quantitative and qualitative estimation of enzyme can be done by using several approaches such as Thread cutting method, Filter paper collapsing method, Spectrophotometric method, Flat band method, Branch and swain method, CMC method and so on. Among all of them spectrophotometric method is the most applicable approach for the quantification of cellulase enzyme. The growth can be monitored by studied their growth kinetic parameters such as cellulase activity, amount of substrate, and protein activity through a graphical representation of pre-treated biomass concentration in different phases. The kinetic study, catalytic activity, and optimization through different parameters may increase the production of the enzyme during hydrolysis by using fugal strain species. Thus, this abstract intends to describe potent microorganism that are more appropriate, fast-growing cells, time-saving, economically effective and their growth estimation approaches. Basis of future scenario the bioethanol production is eco-friendly and cost-effective by using this approach.

Keywords: Cellulase enzyme, microorganism cultivation, growth estimation, spectrometric approach, Growth kinetics.

Introduction

Increase of population worldwide may result as rapid demand for production of abiotic and biotic resources to fulfill our day to day life desires. Consumption of fuel mainly for transportation purposes is one of the major issues as concerned with population and urbanization increases. Biofuel emerges as an alternative source for fuel. It can be extracted from different sources by applying different approaches. Lignocellulosic feedstock is one of the good option for the production of biofuel due to its richness in different types of carbohydrates consist of cellulose, lignin and hemicellulose and due to it's abundantly avability. Accompying some other advantages as well that is easy availability, less expensive, less manpower requirement and ecofriendly.(Anil S. Prajapati.et.al.2020).

Cellulase is an enzyme used for the Saccharification of cellulose into cellulase for the production of bioethanol from lignocellulosic biomass. Lignocellulosic biomass primarily consists of major components, such as cellulose, hemicellulose, and lignin. Cellulase enzyme hydrolyzes β -1, 4 linkages in cellulose chain which converts cellulose into glucose monomer. This conversion can be enhance by using different microbial strains which increase the rate of conversion of cellulose into cellullase. Goind through the several literatures it is found that Aspergillus *niger* is the most potent and easily cultivated microbial strain to obtain the growth of cellulase enzyme. The glucose is further converted to bioethanol using *enzyme* in ethanol fermentation steps thus the process economics is highly dependent on the availability of free fermentable sugar into hydrolysate. The greater the efficacy of enzymatic hydrolysis, the higher the amount of sugar will be leading ultimately to a higher yield of bioethanol in the process. The conversion of a large amount of cellulose to glucose ultimately enhances the production rate of bioethanol in a microbial fermentation system. The overall production cost of bioethanol may be reduced by direct use of cellulase enzyme thus improving the fermentable sugar availability in the process. Cellulase enzymes can be produced from various microorganisms and some animals also. Several bacterial and fungal strains have been reported to enhance the production of hydrolyzed biomass with high sugar loading in the hydrolysate broth. The biological conversion of biomass using pure cellulase enzyme is the utmost required step in reaching an economical model of ethanol production though faced with severe challenges of process-related complications such as heterogeneity of broth, odd mass, and heat transfer, changing rheology of broth during progressed microbial growth stages. Also, the control of process variables is another aspect of process design and development of

industrial-scale production of cellulase enzyme for application in the bioethanol industry. Thus, this paper intends to describe the major technical bottleneck in the industrial realization of cellulase enzyme production and the way forward. By using huge amount of cellullase enzyme directly it is beneficial for its specificity and also doesn't produce undesirable byproducts (Fan et.al,1987) (Lee).

Materials and Methods

Production of biofuel broadly divided into five major steps listed below:-

Collection and chopping of plant feedstock:-

Sorghum leaves consist of cellulose, hemicellulose and lignin in it with very huge concentrations in which quantity of cellulose is extra comparatively. Our study is based on sorghum leaves and their compositional analysis as concerned with cellulose analysis.

Pretreatment of lignocellulossic biomass is the first step for the formation of bioethanol. The main motive of pretreatment is to breakdown of carbohydrate polymers and increase the porosity of feedstock for a good amount of production.

Pretreatment of plant feedstock:-

Pretreatment of lignocellulossic biomass is the first step for the formation of bioethanol. The main motive of pretreatment is to breakdown of carbohydrate polymers and increase the porosity of feedstock for a good amount of production. These can be achieved by four conventional methods. Among the two of them is most favorable form that is Physico-chemical Approach is the combination of two approaches physical and chemical through which cellulose, hemicellulose can be dissolve easily and lignin structure can also be degraded results an improved availability of the cellulose for hydrolytic catalyzed enzymes can be obtained (A T W M Hendriks 1 et. al, 2008) and Biological Approach is the most affordable pretreatment method comparatively with all other methods. Other approaches require expensive instruments which have high energy consumption requirements results in increase the cost of the pretreatment method .Moreover chemical pretreatment produce toxic products, toxic substances which will be interfering during microbial fermentation (Zahid Anwara. Et.al, 2014) technique utilizes different types of fungi, bacteria and other microorganism which degrade cellulosic of

feedstock. Advantage of using these technique economically feasible, less energy consumption rate and increased amount of yields without producing any toxic byproducts results as decline rate in air pollution (AshwaniKumar . et.al, 2008).

Enzymatic Hydrolysis of feedstock:-

It is required to change to conformation of biomass such as size, structure and its chemical composition. The hydrolysis process can be improved significantly by elimination of cellulose, lignin and hemicellulose, increase of porosity, and reduction of cellulose crystallinity by using pretreatment approach. Cellulose hydrolysis can be done by using acids and enzymes simultaneously for huge amount of yield. Acid hydrolysis was a conventional method which includes dilute acid can be used under conditions of both high temperature and pressure whereas concentrated acid can be used at lower temperature and atmospheric pressure .Factors affecting hydrolysis of cellulose such as porosity of LCB, cellulose fiber crystallinity and amount of lignin and hemicellulose. By using huge amount of cellullase enzyme directly it is beneficial for its specificity and also doesn't produce undesirable byproducts. After inoculating *Aspergillus Niger* fungus into lignocellulossic biomass directly, it enhances rate of yield of cellulase enzyme which ultimately increase the sugar production in biomass.

Utilization of microorganism to utilize and production of cellulase enzyme

Cellulase producing fungus were isolated from soil and identified as *aspergillus Niger*. Production of cellulase required two major constituents such as a potent strain and large amount of carbon source as a substrate. Under Submerged Fermentation, an optimized condition required to enhance production rate. Sorghum biomass contains cellulose in large proportion and it contains glucose used as a substrate in which enzymatic reactions carried out. With the help of microbial hydrolysis complex structure of cellulose chain can be broken and converted into cellulase enzyme.

Results and discussion-

Observation Table Number 1

S.No.	Biomass-Residue	Cellulose	Hemi-cellulose	Lignin
		% w/w		
1	Green Sorghum leaves	44.6 %	27.1%	20.7%

Observation Table Number 2

Media Composition of *A.Niger*

S.No.	Components	Concentration	1 lt. Per ml
1	Dextrose	40 gm	1 lt.
2	Peptone	10 gm	1 lt.
3	Agar	15 gm	1 lt.
4	Distilled water	1000 ml	1 lt.

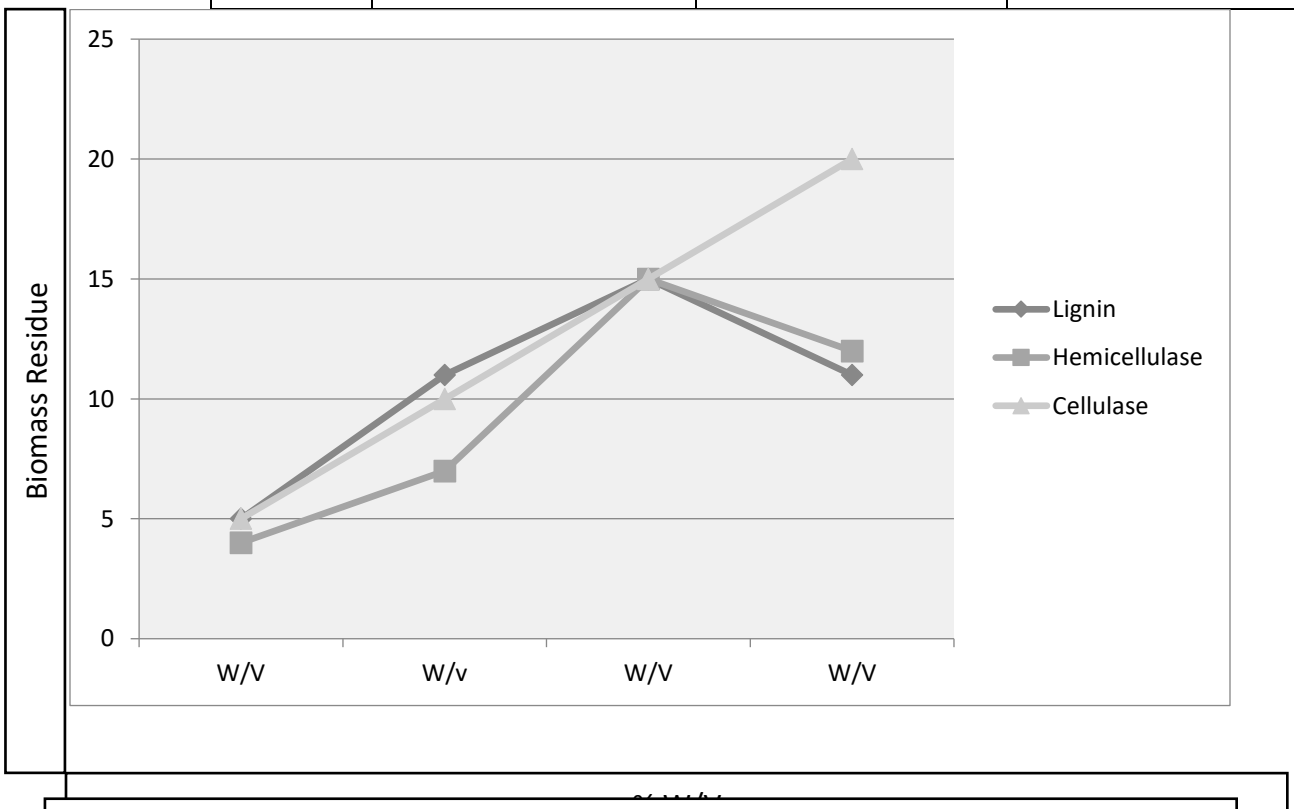

Fig 18. Amount of LCB in sorghum (Growth)

References

1. *J. Iqbal1, 2. I. (February 13, 201). Isolation of Aspergillus niger Strains from Soil and their Screening and. *Journal of Applied Environmental*.
2. 2019. (2019). Different Pretreatment Methods of Lignocellulosic Biomass for Use in Biofuel Production.
3. A T W M Hendriks 1, G. Z. (2008 Jul 2). Pretreatments to enhance the digestibility of lignocellulosic biomass. *ioresour Technol*.
4. a, V. B. (23 May 2011). Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances*.
5. A.G.PereirabFranciscoMaugeri-Filhoa, R. M. (2013). Cellulase production from a new strain Acremonium strictum isolated from the Brazilian Biome using different substrates. *Bioresource Technology*.
6. Abdullah1, 2. H. (2018). Optimization of Cellulase Production by Aspergillus niger ITBCC L74.
7. AbigailGregoryG.PaulBolwell. (1999). *Hemicelluloses.*
8. al, M. D.-A. (2021). Recovery of Agricultural Waste Biomass: A Sustainability Strategy for Moving Towards a Circular Bioeconomy.
9. Alejandro Tellez-Jurado . (29 August 2011). Isolation of Bacteria with Antifungal Activity against the Phytopathogenic Fungi Stenocarpella maydis and Stenocarpella macrospora. *International Journal of Molecular Sciences*.
10. Anil S. Prajapati Ketankumar Joitaram Pancha. (n.d.). Review on Cellulase and Xylanase Engineering for Biofuel Production.
11. AshwaniKumarSatyawatiSharma. (July 2008). An evaluation of multipurpose oil seed crop for industrial uses (Jatropha curcas L.): A review. *Industrial crops and products*.
12. BaribMaurizioPetrucciolia, S. (August 2019). Cynara cardunculus a novel substrate for solid-state production of Aspergillus tubingensis cellulases and sugar hydrolysates. *Biomass and bioenergy*.
13. Caitlin S. Byrt, C. P. (16 December 2010). C4 Plants as Biofuel Feedstocks: Optimising Biomass Production and Feedstock Quality from a Lignocellulosic Perspective. *Journal of Integrative Plant Biology*.

14. Chris Somervill, *. H. (November 12, 2014). Feedstocks forLignocellulossic Biomass. *science.1189268*.
15. Edem Cudjoe Bensah 1, 2. a. (2013). Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations.
16. Elizabeth E. Hooda, 1. (2016). Plant-based biofuels.
17. Fan, L. T., Gharpuray, M. M., & Lee, Y. H. (1987-01-01). *Cellulose hydrolysis. Biotechnology monographs. Volume 3.*
18. Farid Talebnia, D. K. (23 December 2009). Production of bioethanol from wheat straw: An overview on pretreatment,. *Bioresource Technology*.
19. FaridTalebniaDimitarKarakashevIriniAngelidaki. (July 2010). Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. *Bioresource Technology*.
20. Francesco Cherubini, A. H. (23 May 2011). Chemicals from lignocellulosic biomass: opportunities, perspectives, and potential of biorefinery systems. *Biotechnology & Applied Microbiology*.
21. Han Wei, .. T. (2017). Sustainable Energy Technologies & Sustainable Chemical Processes. In *Lignocellulosic Biomass.*
22. Harsha D. Magurudeniya, O. l. (n.d.). Use of ensiled biomass sorghum increases ionic liquid pretreatment efficiency and reduces biofuel production cost and carbon footprint. *Green Chemistry*.
23. Iqbal, J. (2015). Isolation of Aspergillus niger Strains from Soil and their Screening and Optimization for Enhanced Citric Acid Production using Cane Molasses as Carbon Source.
24. Kanak Raj, C. K. (2018). Kanak Raj, Chandraraj Krishnan∗. *t ScienceDirect*.
25. Kim, M., & Day, D. (2010). Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. *Journal of Industrial Microbiology and Biotechnology 38(7):803-7*.
26. Kyoung Hyoun Kim 1, I. S.-J. (2014 Feb). Bioethanol production from the nutrient stress-induced microalga Chlorella vulgaris by enzymatic hydrolysis and immobilized yeast fermentation. *Bioresour Technol*.
27. L. T. Fan, Y.-H. L. (n.d.). *The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis.* Fan, L. T. (1982). [Advances in Biochemical

Engineering/Biotechnology] Microbial Reactions Volume 23 || The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. , 10.1007/3-540-11698-2(Chapter 4), 157–187. doi:10.10.

28. Lapuertaa, M., & ∗, J. J. (6 August 2002). Kinetics of devolatilisation of forestry wastes from. *Biomass & Bioenergy*.
29. Lee, L.-t. F.-H. (n.d.). *Acid Hydrolysis of Cellulose.* Biotechnology Monographs book series.
30. Masahide Yasuda, K. T. (2013). *Effectiveness of Lignin-Removal in Simultaneous.* INTECH.
31. Mensah, E. C. (2018). Emerging Physico-Chemical Methods for Biomass Pretreatment.
32. Mtui, G. Y. (May 2009). Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *AFRICAN JOURNAL OF BIOTECHNOLOGY*.
33. Mtui, G. Y. (May 2009). Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *AFRICAN JOURNAL OF BIOTECHNOLOGY*.
34. Mustafa Balata, ,. H. (28 January 2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science*.
35. Nagaiah Premalatha1, N. O.-W. (08 October 2015). Optimization of cellulase production by Enhydrobacter sp. ACCA2 and its application in biomass saccharification. *Tianjin University of Science and Technology, China*.
36. Oberoi HS, V. P. (2010). Ethanol production from orange peels: two-stage hydrolysis and fermentation studies using optimized parameters through experimental design. J Agric Food Chem 58(6):3422-9. *SGD*.
37. Oscar J Sánchez 1, C. A. (2007 Dec 26). Trends in biotechnological production of fuel ethanol from different feedstocks. *NCBI*.
38. Ozarska1, N. N. (20 March 2018). Comparison of two dye uptake measurement methods for dyed wood. *CrossMark*.
39. Parameswaran Binod *, R. S. (26 November 2009). Bioethanol production from rice straw: An overview. *Bioresource Technology*.
40. R P Chandra 1, R. B. (26 May 2007). Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics. *Springer-Verlag Berlin Heidelberg*.
41. Sarkar J, e. a. (2012). Yeast mitochondrial leucyl-tRNA synthetase CP1 domain has

functionally diverged to accommodate RNA splicing at expense of hydrolytic editing. J Biol Chem 287(18):14772-81. *SGD*.

42. Shuangqi1, T. (2011). Determination methods of cellulase activity .
43. Talebnia, F. (n.d.). Production of Bioethanol From Wheat Straw: An Overview on Pretreatment, Hydrolysis and Fermentation.
44. Teter, C. J. (May 2005). Prevalence and Motives for Illicit Use of Prescription Stimulants in an Undergraduate Student Sample. *Journal of American College Health*.
45. Thanaporn Laothanachareon 1, B. B. (2022). Profiling multi-enzyme activities of Aspergillus niger strains growing on various agro-industrial residues.
46. VaraPrasade, O. S.-I. (May 2020). Production of biofuels from sorghum.
47. William L. Rooney, J. B. (19 September 2007). Designing sorghum as a dedicated bioenergy feedstock. *Biotechnology & Applied Microbiology*.
48. Y.C. Sharma a, *. B. (20 February 2008). Advancements in development and characterization. *Science Direct*.
49. Yaser Dahman, .. A. (2019). An introduction to biofuels, foods, livestock, and the environment.
50. Ye Sun, J. C. (31 October 200). Hydrolysis of lignocellulosic materials for. *Bioresource Technology*.
51. ZahidAnwarabMuhammadGulfrazbMuhammadIrshada1. (April 2014). Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *Journal of Radiation Research and Applied Sciences*.

International Journal of Advanced Research in Biotechnology and Nanobiotechnology
Vol. 3, Issue I, July 2022 **ISSN: 2582-3310**

Xylanase Enzyme Production By Using Microbes For The Biofuel

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Abstract

As we know the biotechnology is one of the focused scientific field where all the highest development processed during the last few decades. It is the grouping of biological, physical and engineering, technical sciences in order to complete the hi-tech application using biological system. So for Processing the above we use the xylanase enzyme (Endo-1, 4-β-), which is a class of biological enzymes which used to degrade the linear polysaccharide i.e. xylan into xylose, thus it will help in breaking down the one of major components of plant cell walls i.e. https://en.wikipedia.org/wiki/Hemicellulose hemicelluloses which is a major component of lignocellulosic biomass and thus also help in breaking of cell-wall that play major role in bioethanol production. Xylanase have the property to process the pre-bleaching and the bioconversion of the lignocellulosic biomass into the usable feedstock as well as usable nutrient. As we know the now days the industries are looking for the alternate and best eco-friendly approach so by using the xylanase for biofuel production is one of the major step towards the biogreenery approach. In this paper reviewed many of the research paper then get to know that Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, crustaceans, seeds. By following the last few recent research obtain the some of best microbial sources for the xylanase production were listed these are Micrococcus sp., Bacillus sp., Aspergillus Flavus & A. niger, Fusarium sp. In yield of Xyalanase the fungal strain consider i..e..*Fusarium sporotrichoides* by the SDA mediun which confront the growth. In yield of Xylanase the few parameter were determine i..e.. the fermentation method, carbon & nitrogen source, pH, temperature, ionic-effects, incubation time, aerations, and species. Kinetic study, catalytic activity and optimization through different parameters may increases or help in the production of enzyme during hydrolysis by using fugal strain species. The production of xylanase on large number of scale is expensive because it requires a huge amount of enzyme. It Concluded that using of microbes are more appropriate and best approach for biofuel production, fast growing cells, time saving and economically effective.

So on the basis of future scenario the bioethanol production is ecofriendly and cost-effective by using the enzymatic approach.

KEYWORDS: Lignocellulosic, Fusarium species, optimization, kinetic growth, xylanase enzyme, biofuel.

INTRODUCTION

In today's world the scenario regarding the biofuel is totally desirable production for the better conditions of the atmosphere as well as ecological best. Biotechnology consider one of the main technical field where most of the maximum progress occured during the last many of the decades it has been taken place. It will be the combination of the biological, physical and an engineering, technical innovation in order to achieve the scientific application by using biological system.(Uma Shankar Prasad Uday.et.al.2015). We know it's a desirable amenities for the living world and as well as the high demand on the industrial scale for the ecological demand.

Now days the amount of utilization biofuel is very high in the both level that is domestic and industrial stage as above mentioned. The general significance of the fuel is like that it burned to provides the nuclear energies heats, and powers to fulfill the energy consumption demand. The material like the coal, wood, oil and gas could make available the heat while it will be burned. The substances like methanol gasoline, diesel, propane, natural gas, and hydrogens are type of fuel. (Hall..et.al2007).

A fuel can be any material which could be made up to act in response with the other substance so that it be able to release the required energy that can be used to work.(schobert..et.al.2013).The concept of the fuel can be applied to those materials which can be competent of the releasing the chemical energy however it has been also apply to the other source of the energy in the face of the heat and nuclear energy (via nuclear fission and nuclear fission).(Hall..et.al.2007).

As considering the lignocellulosic biomass just because of easily available in the agriculture waste found inexpensive, and also the large amount the waste found in our agriculture production. (Anil S. Prajapati.et.al.2020).

The Lignocellulose which is consider important source for the production it is composed with the three major factor: cellulose, hemicelluloses and lignin.

Biomass category	Cellulose	Hemicellulose	Lignin
		% W/W	
Barely	33.8	21.9	13.8
Corn cob	35.0	16.8	7.0
Cotton residues	58.5	14.4	21.5
Rice residues	36.2	19.0	9.9
Sugar cane	40.0	27.0	10.0
Wheat straw	32.9	24.0	8.9

Table 1: standard share of key components of chosen lignocelluloses biomass supplies in Preferred unrefined material.(Karolina Kucharska.et.al.2018)

The conversion totally relies very much on major technological innovations which is founded and centered for effective and low-cost enzymes, feed-stocks, and with the considerations in efficient process design.(Anil S. Prajapat.et.al.2020).

In this paper we considering the recent studies of the microbes like bacteria, yeasts as well as filamentous fungi commonly used in all the biotechnological procedure, on the other hand vascular plants, algae along with the animal tissue as well its utilizes. But the recent use of biotechnology have paying attention on all natural yield such as enzymes, antibiotics, and hormones. (Uma Shankar Prasad Uday.et.al.2015).

The enzyme considered for the production process for biofuel that is xylanase enzyme because it can be the cheaply and easily available.

Xylanase enzyme (Endo-1, 4-β-), is a class of biological enzymes which used to degrade the linear polysaccharide i.e. xylan into xylose, thus it will help in breaking down the one of major components of plant cell walls i.e. https://en.wikipedia.org/wiki/Hemicellulose hemicelluloses which is a major component of lignocellulosic biomass and thus breaking of cell-wall play major role in bioethanol production. (Asish Mandal.et.al***.2015*).** Xylanase produced by the fungi,

bacteria, yeast, marine algae, protozoans, crustaceans, seeds. Last few recent research study some of best microbial sources for the xylanase production were listed these are *Micrococcus sp., Bacillus sp., Aspergillus Flavus & A. niger, Fusarium sp.*

In this we consider the fungal strain i..e, *Fusaarium Sporotrichoides* species because going through many of the research paper this fungal strain found suitable for the biofuel production.

Materials And Methods

The methodology used for the production process for the production of enzyme is mentioned below-

- **Collection of Biomass :**

  The collection of biomass is a major part for the production process of bioethanol because the collection of materials process impact role in production of biofuel and as well as enzyme production for the biofuel. The collection procedure of biomass can be done in few ways for production process these are :-

  Agro-Waste Selection –

  As we know generally bioethanol can be produced from various types of agricultural sources such as Sugarcane, Castor, Lignocelluloses biomass ,Sunflower, Maize, Wheat, Molasses, Agricultural waste, Sorghum, Soybean but for bioethanol production in this paper consider the **Green Rice Straw** because it contains cellulose, hemicellulose and lignin which mainly consider the major component for the production process for bioethanol.

  Biomass Substrate Separation –

  The separation process define the way for obtaining the desire product so after collecting substrate i..e.., the rice green straw leaves which keep it in natural sunlight for few days i..e., approx 2-3 days to get it in dry form for procedure.

  When the leaves become dry in accurate way then after this it will grind all the dried leaves to get it in powder form properly.

❖ **Pre-treatment Method :**

The lignocellulosic biomass consider the most resistant to the biological and as well as chemical breakdown for the preffered method and this procedure also called as biomass sedition. The numerous factor such as crystalline structure of cellulose, and the amount of lignifications and the structural heterogeneity as well as the density of the cell wall constituent which mainly liable for biomass sedition and also moniterd that should be overcome for important consumption of the lignocelluloses biomass feedstock's. (Julie Baruah.et.al.2018).

The pretreatment method can be approached for solubilization along with the separation of more than one components of the lignocelluloses biomass. The lignocelluloses biomass component made up of the matrix of the cellulose and lignin. The further step has be in use to create the solid mass more easy towards get to the chemical and natural treatment. (Bedadyuti Mohanty and Ismail Ismail Abdull.et.al.2019). There are few most important factors for a valuable pretreatment has a number of objectives these are –

1. It will reduce the crystallization as well as increase the surface area of the cellulose meant for the enzymatic digestion process.
2. This method also solubilize the hemicellulose or lignin also.
3. This method avoid the loss of sugar during the process.
4. It will also minimize the formation of unwanted lignocelluloses derived inhibitors.
5. The pretreatment method will also help in minimize the energy and demanding capital costs.

(Daehwan Kim.et.al.2018)

The Pretreatment method generally perform into four ways for the desired production of the biofuel these are-

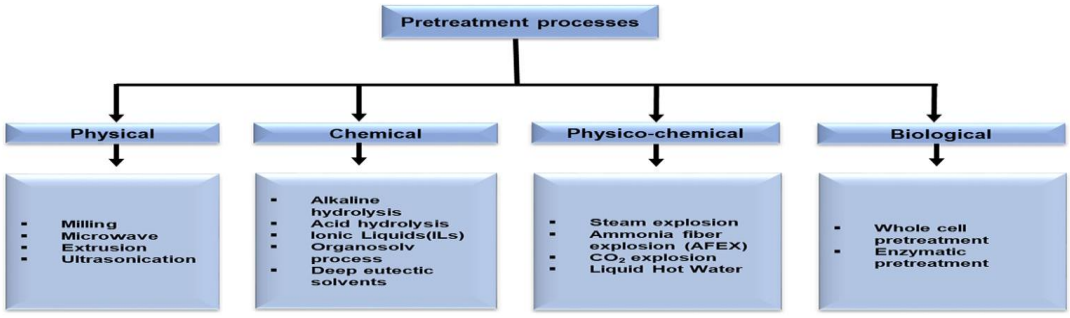

Figure – Types of Pretreatment Method (Julie Barual.et.al.2018).

But in this paper we were consider only the two main basic method for the desired production of biofuel these are –

- **Physical Pretreatment Method**
- **Chemical Pretreatment Method**

➢ **Physical Pre-treatment Method :**

The physical pretreatment method describe that it consider one of the major step for the breaking of linkage and formation of products in comparison of other pretreatment process. It is step to lighten way in to the raw materials for reducing the particle size and increase the efficiency of the yield. This process also decrease the level of polymerization and also the crystalline of the lignocelluloses. This approach become more easy and effective method and also it is the eco-friendly approach and very rarely any kind of toxic substance obtained. In this physical pretreatment method the size reduction were analysed and estated that the reduction of the softwoods.

The types of the physical pretreatment method which is commonly and majorly used for the production process of the biofuel these are listed below –

⇒ Miling
⇒ Extrusion
⇒ Microwave (The Irradiation)

⟹ Ultra-Sonications

(Julie Baruah.et.al.2018).

- **Chemical Pre-Treatment Method:**

The chemical pretreatment had been occur by the using alkaline and acidic method for the biorefinery process. It is mostly used and consider good pretreatment method in comparing the physical and biological pretreatment method and also chemical method very effective and also increases the chances of the biodegradation of the complex material. This method approached for obtaining the good productivity of the biofuel in laboratory level as well as industrial level. The mainly used chemicals for doing the improvement of performance of all the residues which taken as a agricultural material in production. The chemical used they are the Sulfuric acid (H2SO4), hydrochloric acid (HCL), acetic acid (CH3COOH), sodium hydroxide (NaOH), potassium hydroxide (KOH), lime (Ca(OH)2), aqueous ammonia (NH3·H2O), and hydrogen peroxide (H2O2). This pretreatment method optimized the study for production and help in bereaking the complex bond between the biomass substrate and help in degrading into desire form. (Amin et al. AMB Expr.2017).

❖ **Micro-Organism Cultivation Method :**

In this paper the suitable microbial cultivation method is being used for the production of enzyme i..e.., xylanase for degredation of complex material in biomass for the production of the biofuel. Thus the xylanase backbone has been composed of the β-1, 4-linked D-xylopyranosyl.
(Asish Mandal.*et.al.2015*).

In this paper we consider the fungal strains for the production and it is the Fusarium Sporotrichoides Species because this strain would found best and major source for the production of xylanase and also well showing the enzymatic activity.

The F.sporotrichoides were obtained or isolated from source is soil sample method. The soil sample was collected from the garden areas and the suspension culture was prepared i..e.., by dissolving the 5gm of soil sample in each 10 ml means we totally required 100 ml of distilled water into flask with the sterilized conditions after this all the test tubes were centrifuged till the soil mixed into the distilled water. After this it will keep for one day in 30℃ that is normal room temperature and also prepare the broth culture of agar medium.

For the media making the medium and the broth culture required for the Fusarium strain that is prepared by the preparing the SDA (Sabourauds Dextrose Agar) for the *Fusarium sporotrichoides* we need 40 gms of dextrose in per litter, peptone 10 gms in per litter, agar 15 gms in per litter, and distilled water 1000 ml. After preparing the SDA broth medium measure and maintain the pH balance between 5.6 , and keep it for the autoclave at 15 lbs pressure at 121℃ temperature. After this the pouring and plating method will be done on 5 petri-plates as. So after this the spread plate technic were done by taking and inoculation done from soil sample method in SDA media plate.
After all the procedure the media plates was kept on incubation for 3-4 days at 30℃ temperature. After the obtaining the growth of fungi the pure cultured of isolates were obtained by the repeated sub-culture on SDA. And after this the conformation for the growth of *Fusarium sporotrichoides* were done by preparing and using the congo-red method for the xylanase production.(Shankar et al RJLBPCS 2018).

❖ **Hydrolysis And Saccharification Method :**

In the hydrolysis method the generation of the by-product which was obtained by the pretreatment is powerfully dependent on the taken feedstock and as well as the pretreatment method. substance that could be active as inhibitor for the microorganisms with the includeness of phenolic compounds and along with other aromatics, aliphatic acids, furan aldehydes, inorganic ions, and bioalcohols and along with other fermentation products.
By using the agriculture residues it signified that hydrolysis is easy to breaking the linkage bond in compare the softwood residues. Hydrolysis of the cellulose component can be catalyzed through using strong inorganic acids and hydrolytic enzymes for obtaining the biofuel. By approaching the acid hydrolysis for production requires the several conditions but if we talk about the enzymatic hydrolysis then the enzymatic approach for the hydrolysis is often considered the most potential and effective method for upcoming scenario in the term of the biofuel production. (Jönsson.et.al. Biotechnology for Biofuels 2013).
As from the last few recent research the examine conditions for the xylan enzyme have been report to lie within the 50℃ temperature and pH lie between the 4 to 5.
(Bedadyuti Mohanty and Ismail Ismail Abdullah.et.al.2019).
After the performing the hydrolysis of lignocelluloses polysaccharides, The lignin component remains as a solid deposit in the substance , even though a negligible part is degraded to the

phenolics and also in many aromatic compounds. The sugars processed from the hemicelluloses could be report for a considerable part of the total sugar and it is advantageous that they are included in the succeeding fermentation step. The monosaccharides that obtained all the way through the hydrolysis process and then fermented by the microbial catalysts to the production of the preferred product.(Jönsson.et.al. Biotechnology for Biofuels 2013)

But if we talk about the saccharification method then in compare to the hydrolysis approach this method then the Saccharification process consider the vital step for the bioethanol production where all of the complex carbohydrates will be converted into simple monomers. So on this basis the hydrolysis mostly used method in the biofuel production. (Bedadyuti Mohanty and Ismail Ismail Abdullahi –et.al.2019).

- **Fermentation**

The fermentation method is the down-streaming footstep approach for the production processes in which the microbial metabolic activity takes place which converts soluble sugar into alcohol (Bioethanol). Xylanase can be produced by using different types of fermentation techniques. However, solid-state fermentation and sub-merged fermentation is report to have the finest result fermentation culture process considered for the bestest approach for the development and it is also the most broadly studied at present.

- **Solid-State Fermentation (SSF):-**

As per our work, Production of xylanase using *Fusarium* Species can be processed out under the solid-state fermentation due to some advantageous factor i.e., it can be capable of easily scaled up to the pilot scale or industrial level using newly that make-believe mechanization techniques which do not suffer from heat mass transfer as compare to other fermentation techniques. (L.Motta.,et.al 2012). Ethanol fermentation can be takes place in the occurrence of oxygen and it enhance the cell biomass that result into the invention in the large amount [Babbar and Oberoi, 2014]. It is the most convient and industrial based process at an optimum temperature and ph. [Devi.et al.,2011,] [Gedela.et al.2017].

- **Method of quantification of enzyme produced for biofuel production**

- **Xylanase activity determination method :**

According to the study or going through the review paper of the (Shankar et al 2018) the activity of the xylanase enzyme could be observed once and it will be produced and obtained after the performing all these determination and it will be done by the several methods by going though many of the last recent research or review papers and some of the found suited these are the mentioned below :-

1. Temperature and the pH effect for the determination.
2. Using the inducers and agitation method for the determination.
3. The incoloumn size of the microbes and the growth time.

Once an enzyme can be produced, activity of enzyme can be measured after 24hr of production. Quantitative and qualitative estimation of enzyme can be done by using several approaches such as Thread cutting method,Filter paper collapsing method, Spectrophotometric method, Flat band method, Branch and swain method, CMC method and so on. Among all of them spectrophotometric method is the most applicable approach for the quantification of cellulase enzyme. The growth can be monitored by studied their growth kinetic parameters such as xylanase activity, amount of substrate, and protein activity through a graphical representation of pre-treated biomass concentration in different phases. The kinetic study, catalytic activity, and optimization through different parameters may increase the production of the enzyme during hydrolysis by using fugal strain species.

❖ Results And Discussion –

Observation Table No. 1 – Composition Content of Biomass-

S.No.	**Biomass-Residue Type**	**Cellulose**	**Hemi-cellulose**	**Lignin**
		% w/w	**% w/w**	**% w/w**
1.	Green-rice straw	35 %	21%	11.5%

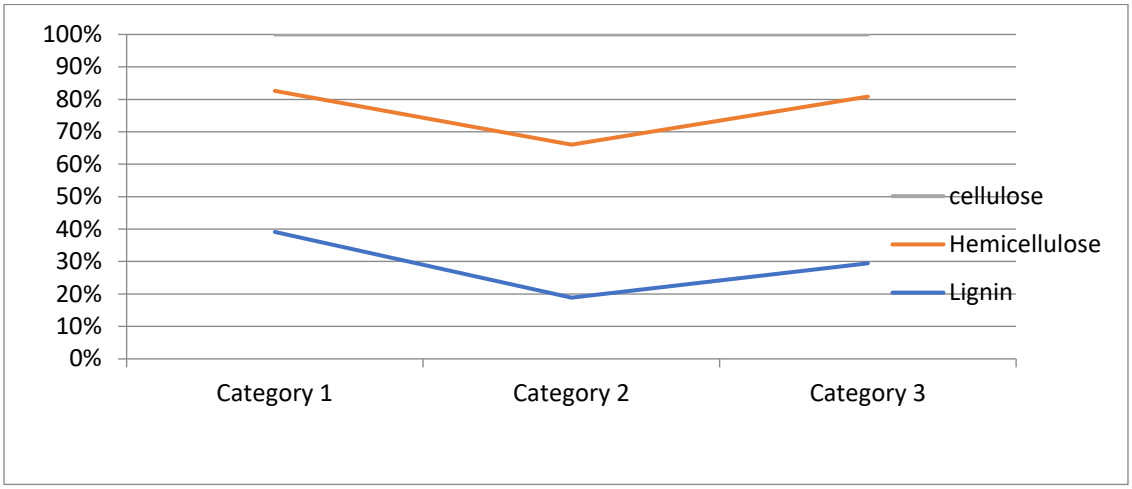

Figure :1 Chart Presentation

Observation Table Number 2 - Before Pre-Treatment pH Determination of sample

s.no	Pretretment-Method At HCL Conc.	Biomass Concentration	Acid Conc. [H2SO4]	Time	Temp.	Normal ph of Kcl Ph at 4,7,9
1	5 ml	10 gm	20 ml	35 min	170 ℃	5.5
2	20 ml	10 gm	5 ml	35 min	80 ℃	5.5
3	5 ml	10 gm	5 ml	15 min	80 ℃	5.5
4	12.5 ml	17.5 gm	12.5 ml	25 min	125 ℃	5.5

Observation Table Number 3- After Pre-Treatment pH Determination for sample

S.No.	Pretreatment Method At HCL Conc.	Biomass Concentration	Acid Conc. [H2SO4]	Time	Temp.	Normal pH of Kcl pH at 4,7,9
1	5 ml	10 gm	20 ml	35 min	170 ℃	0.74
2	20 ml	10 gm	5 ml	35 min	80 ℃	0.42
3	5 ml	10 gm	5 ml	15 min	80 ℃	0.75
4	12.5 ml	17.5 gm	12.5 ml	25 min	125 ℃	0.52

Observation Table Number 4 - Media Composition

S.No.	Components	Concentration	1 lt. Per ml
1	Dextrose	40 gm	1 lt.
2	Peptone	10 gm	1 lt.
3	Agar	15 gm	1 lt.
4	Distilled water	1000 ml	1 lt.

Observation Table Number 5- Pre-Treated Biomass Quantitation For xylanase production

S.No	Pre-Treated Biomass Residue	Microorganism	Condition		Glucose Release	Quantity of xylanase enzyme production
			Ph	Temperature		
1.	Green- Rice straw	*Fusarium Sporotrichoides*	5.6 / 30°C		42.-43.1 %	16.8-17.9%

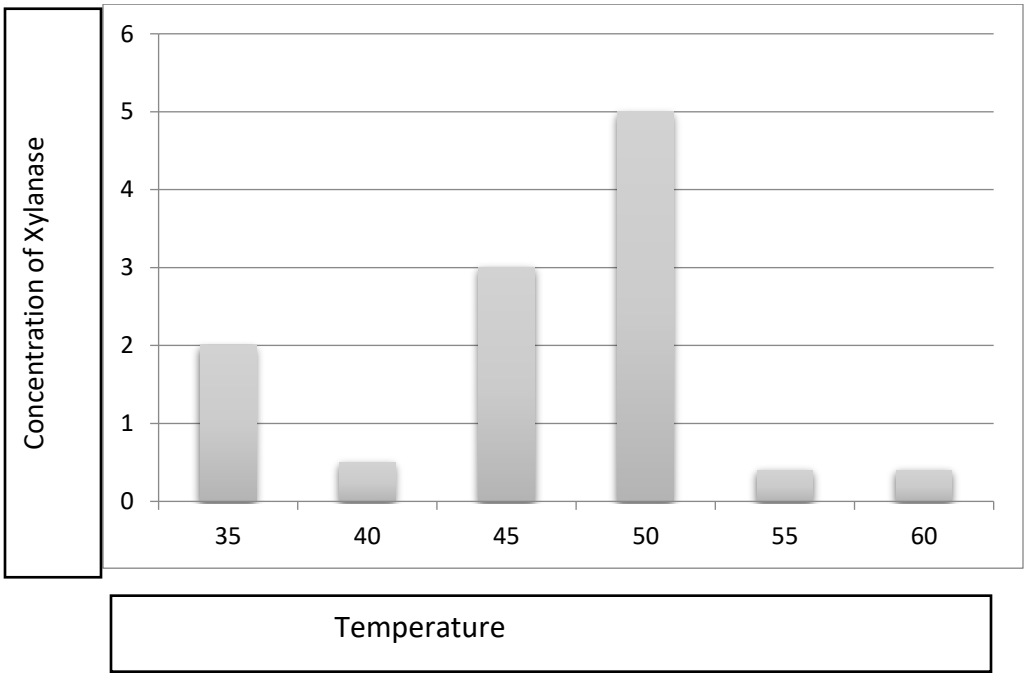

Figure 2: Graphical Presentation of Table No.5

CONCLUSION

We are very friendly that current scenario where the focused being to use the all the raw materials that obtained as a waste from the agriculture field and in today technology and development to make something bio-friendly product to protect our environment and atmosphere.

So, as we all know very well the agriculture waste is releasing in very high amount and also in trending for the today's recent research it fascinated and found very effective and environment friendly for the industrial production Biofuel.

This paper try to attempt a research to identify a number of solutions to set up a sustainable production. Here we tried to use the rice straw waste for our biomass material and perform with the pre-treatment that done by including both after and before by determine their pH in both condition. Then the including the production using hydrolysis that is enzyme method to obtained the biofuel by performing micro-organism cultivation method and afterward it result into the result got enzyme that is xylanase production for the treatment and the filteration and distillation method perform to know.

And using this method by including the enzymatic method result into the environmentally friendly and the safe energy resource, and along with the mainly effective means for avoiding all the negative impacts of the biofuel production that trying through the providing fuel from the agricultural residues and the lignocellulosic compound in as short period which can be as possible with the considering the economical values and the cost effective for the coming times as well a future scenario.

FUTURE PROSPECTS

- Using the lignocellulosic biomass that obtained from the agriculture waste for the production process is consider the beneficial in the production and as well environment point of view.
- Using the agriculture waste for production technology is also consider into a cost estimation and benefit for the future scenario development and also found the very effective for the industrial point of view.
- The enzymatic hydrolysis consider the cost-effective as well as the it also added the increasement for the new demand by-product.

- The enzymatic process also added and increased the nutrional and functional value based for the industrial production.
- It also possible that using lignin cellulose and hemicelluloses give the new possibilities to the bio-refinery world in the industries level.
- Using the renewable sources as a lignocelluloisc biomass for the production of biofuel also decreasd or remove the demand for the food crops for any of the development.

REFRENCES

1. Annamalai, N., Thavasi, R., Jayalakshmi, S., & Balasubramanian, T. (2009). Thermostable and alkaline tolerant xylanase production by Bacillus subtilis isolated from marine environment. Indian Journal of Biotechnology.
2. Xylanase and their industrial application , Dushyant Kumar1, Smita S. Kumar2, Jagdeesh Kumar3, Om Kumar4, Shashi Vind Mishra4, Rahul Kumar5 and Sandeep K. Malyan4.
3. Abdel-Satera,M.A.,andEl-Said,A.H.M.(2001).Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. Int.Biodeterior.Biodegrad. doi:10.1016/S0964-8305(00) 00113-X .
4. Abdullah,R.,Nisar,K.,Aslam,A.,Iqtedar,M.,andNaz,S.(2014).Enhanced production of xylanase from locally isolated fungal strainusing agro-industrial residues under solid-state fermentation. *Nat.Prod.Res.*,1006–1011.doi: 10.1080/14786419.2014.968157.
5. Dodd D, Cann IKO (2009) Enzymatic deconstruction of xylan for biofuel production. Glob Chang Biol Bioenergy 1:2–17.
6. Filho EXF (1998) Hemicellulase and biotechnology. In: Pandalai SG (ed) Recent research development in microbiology. Research Signpost, Trivandrum, pp 165–176.
7. Reddy, C.A.(1994). Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast ofIndia. Botanica Marina 37: 515-523.
8. Thorn, G. (1993). The use of cellulose azure agar as a crude assay of both cellulolytic an ligninolytic abilities of wood inhabiting fungi. Proceedings of the Japanese Academy of Science 69: 29-34.
9. Kumar, R. & Wyman, C. E. Effect of xylanase supplementation of cellulase on digestion of corn stover solids prepared by leading pretreatment technologies. *Bioresour. Technol.* **100**, 4203–4213 (2009).

10. Bajaj, B.K.; Khajuria, Y.P.; Singh, V.P. Agricultural residues as potential substrates for production of xylanase from alkali-thermo-tolerant bacterial isolate. Biocat. Agric. Biotechnol. 2012, 1, 314–320.
11. Swe, K.T. Screening Potential Lignin-Degrading Microorganisms and Evaluating Their Optimal Enzyme Producing Growth Conditions; Chalmers University of Technology: Goteborg, Sweden, 2011.
12. Tahir, A.; Arshad, M.; Anum, F.; Abbas, M.; Javed, S.; Shahzad, M.I.; Ansari, A.R.; Bano, I.; Shah, F.A. Ecofuel future prospect and community impact. In Advances in Eco-Fuels for a Sustainable Environment; Azad, K., Ed.; Woodhead Publishing: Sawston, UK, 2019; Chapter 17; pp. 459–479. ISBN 978-0-08-102728-8.