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The Efficacy of Extract from Various Parts of Blumea Lacera in Preventing and Treating Bacteria (Escherichia Coli) And Fungal (Trichophyton Rubrum) Infections in Humans as Well As Controlling Mosquitoes (Aedes Aegypti) In the Environment.

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Abstract

In Ayurveda, Blumea lacera extraction has been found cast-off in the management of various sympathetic of diseases and is also found to exhibit hypoglycaemic, anti-diarrhoeal, larvicidal, antioxidant and antimicrobial properties. Blumea lacera extraction contain astringent, stomachic, antipyretic, and diuretic, cure bronchitis and fever. To find out the most suitable part of the plant Blumea lacera which will be used for killing the mosquito larvae. How do the plant extraction produce effect on the different stages of larvae, 1st, 2nd, 3rd, and 4th, stages. Different doses of extraction. Blumea Lacera plant, cut in their different parts of plant separately, i.e., leaves, root, stem, and bud and grind them with different solvents like methanol: leaves, root, stem and bud. Distilled water: leaves, root, stem and bud. Benzene: leaves, root, stem and bud, and kept them at room temperature openly to evaporate the excess number of solvents. Mosquitoes larvae (Aedes aegypti) and culture them at room temperature by providing them only filtered rain water and mixture of (3gm pedigree +1gm yeast). Our main focus was on that whether the different solvent extraction is effective against mosquitoes' larvae, if it is then how much effective? Then the most effective extraction was methanolic leaves extraction, it mostly affected the 1st stage of mosquitoes' larvae at 1500 microlitre it killed 90% 1st stage population of mosquitoes' larvae. Methanolic leaves extraction showed antibacterial property, antifungal property and larvicidal property.

Keywords: Blumea lacera, larvicidal activity, antioxidant, antimicrobial, larvae, plant extraction, doses, solvents.

INTRODUCTION

The plant *Blumea lacera* is originated mainly in the Asia roadsides of tropical and sub-tropical zone, especially the Indian Subcontinent and southeast Asia. Nature has provided us various number of medicinal plants. And to get rid of saviour diseases use of plants are going on treatment since the existence of human civilisation. And mostly numbers of prescribed drugs now also derived from plants.

Figure 1. Blumea lacera

Most of the people who can't afford expensive treatment often depends upon medicinal to get rid of disease. Mostly in developing countries more than half of the population depends upon medicinal plant to meet their primary health care needs. It rabi weed of India. It belongs to Asteraceae family. The species *Blumea lacera* has spread widely in India mostly in Maharashtra, Karnataka, Madhya Pradesh, dry region of Uttar Pradesh, and Orissa. *Blumea* lacera is mainly short up to 1-2 meter in height, slim, perennial herb, and often dichotomously and it having strong odour due to the presence of turpentine,
branched and without stipules, and alternate, hairy leaves: Obovate or oblanceolate narrow towards tip and serrated at the margins [Bansaet et al., 2023]. Inflorescence: Heads (Capitulum). Blumea lacera extract contain flavonoids, phenolic compounds, carbohydrate and phytosterols etc. And they have bright yellow flower which are arranged in axillary cymes. And the flowers are spiked in shape and small fruits are appear in December- March. Many reports said that the essential oils that are obtained from its leaves are contain many important phytoconstituents compound like, flavonoids, steroids, tannins, triterpenes, beta-sitosterol, stimaterol-3-O-beta-D glucopyranoside, campesterol etc. In the Ayurvedic medicinal system BL extraction has been used in the curing of various diseases viz: extraction of BL and their isolate have shown anti-oxidant and anti-hyperlipidaemic potential anti-diarrhoeal activity, hypoglycaemic activity, larvicidal activity, antimicrobial properties, anti -cancer activity [Pratap et al., 2006]. Hence it is found that BL extraction contain larvicidal properties which is effective against mosquitos' larvae [Abinaya et al., 2018].

In western side of India, Odisha state there and in Odisha only there is small town present that is called Gunupur. Gunupur is famous for dengue and malaria prone zone. Dengue and Malaria are caused by Aedes species (aegypti or albopictus) and female Anopheles mosquitos. So, our project is basically based on how to control growth of mosquito [Radhakrishnan et al., 2019]. Our main focus is upon the Aedes species of mosquito which cause dengue and they were easily available. Mosquito causes various number of diseases including filaria, malaria, dengue, chikungunya, Japanese encephalitis, and zika virus which are also known as vector born diseases. Nowadays mosquitoes borne diseases have become a major health issue and public headache. And the disease like dengue, malaria they are transmitted in human by Aedes aegypti, Anopheles stephensi. Since long time one of the well-known methods being used for controlling the mosquitoes is the use of man-made synthetic insecticides/pesticides/larvicides.

And with time mosquitoes also developed genetically resistance against man made synthetically insecticides/pesticides/larvicides. Moreover, synthetically prepared larvicides also affect the surroundings by contaminating the soil, water and air [Anoopkumar, et al., 2022]. Therefore, from safety point of view it is urgent need to find out alternative to the man-made synthetic insecticides/pesticides/larvicides, which will be more potent with producing no side effect and biodegradable, and inexpensive too. Hence, ethnobotanical plants which contain all the essential element and rich sources of alternative agents for control of mosquitoes, because they possess biologically active chemicals and which take action against the specific target larvae and it does not cause any harm to the environment and it is eco-friendly [Mukhopadhyay et al.,2015].

Besides all this, it is well known fact that larvicides which are used to kill larvae of any species play most important role in controlling in their breeding habitat. Although, there are various other biological measures are there in vogue with their effective control on larvae stage of mosquitoes and they have been highlighted by using man made synthetic chemical/pesticides with insecticidal properties, such as organophosphate, organochlorine, carbonate and pyrethroids have proven to be most effective method to control the mosquitoes but they also show their effect on other insect pest too and just because of this there is widespread development of resistance by mosquitoes and unwanted toxic, or lethal effect on non-target organisms[Shaalan et al., 2009].

1. Material and method

2.1 Collection of samples: We collected the plant (Blumea lacera) from the back side of department of biotechnology, (Gunupur Odisha), and washed it rigorously under tap running water. After that we dried the sample plant in vacuum oven at 40^0 Celsius for 1hour and then mounted it and send it for analysis at Berhampur University, after we got confirmation, then we unrooted more plants from the same site. And again, we went for washing, thoroughly under tap running tap water. Then we wiped the excess amount of water from the plants, then with the help of knife we cut the different parts of plant (leaves, stem root and bud) separately.

2.2. Chemical and solvents: For the analysis and pharmacological testing laboratory grade reagent were used like CH3OH, purified H_2O, and C_6H_6 used.

2.3. Preparation of sample: Took different parts of sample plant leaves, stem, root and bud and then grind the all the parts of plant with different solvents like methanol: leaves, root, stem and bud. Distilled water: leaves, root, stem and bud. Benzene: leaves, root, stem and bud, with the help of mortar pestle made paste and pour in petri dish and dried at clean and dark place for 1 day. After that all the methanolic solvent, distilled water, benzene evaporated completely, with the help of specula by scratching took out the crude form, weigh it, and methanolic leaves(0.5gm), methanolic root (0.2gm), methanolic stem(0.4gm), methanolic bud (0.4gm). Distilled water leaves (0.4gm), distilled water root (0.3gm), distilled water stem (0.5gm), distilled water bud (0.3gm). Benzene leaves (0.5gm), Benzene root (0.2gm), Benzene stem (0.4gm), Benzene bud (0.2gm). Then add 2ml of distilled water in each solvent extraction and pour all the solvent extraction in 2ml of Eppendorf's tube and refrigerate it.

2.4 Collection of microbial sample: The microbial sample i.e. (Escherichia coli) was collected from microbial Type Culture Collection (MTCC, Chandigarh), then we cultured the E.coli in nutrient agar media and then again, next day we made nutrient agar media autoclave it and then pour in petri plate, allow it to cool down for 1 hour, after solidifying it we do streaking from the master plate and with the help of punched machine we made hole and pour control (purified H_2O + dried CH_3OH extract) and in other hole we added methanolic leaves extraction and wrapped it with parafilm and kept in incubator for 24 hours. After 24 hours it showed antibacterial or antimicrobial activity.

2.5. Collection of fungi: Pure strain of fungi was given by Dr. Polaki Suman and for analysis we cultured the fungi in YPDA (yeast potato dextrose agar) media at room temperature for 5 -7 days. After that with the help of lactophenol cotton blue staining we stain the fungi and observed under microscope at 40X it was confirmed by Dr. Polaki suman that it is only trichophyton rubrum fungi which cause severe skin problem. Then again, we made YPDA media and pour 1ml of fungi culture with the help of pipette, and also put disk of methanolic extraction which was dipped in methanolic extraction for 1 hour under LA (laminar air flow) and wrapped it from parafilm and kept it at room temperature for 2-3 days and after 4 days we observed it shoed antifungal property in methanolic leaves extraction and no changes in control (distilled water + methanol) dried extract.

2.6. Collection of larvae: Mosquitoes (Aedes aegypti) was collected from the backside of department of biotechnology, gunupur, (Odisha). They were present there in huge amount and in different stages like. 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and pupa stage. And allow them to growth at large scale in laboratory at room temperature by providing food (3gm pedigree + 1gm yeast) and filtered rain water, after 1week they were present in large number

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3. Experimental design

Table 1. Table of different parts of plant with their respective solvents.

Root + distilled water	Root + methanol	Root + benzene
Shoot + distilled waster	Shoot + methanol	Shoot + benzene
Stem + distilled water	Stem + methanol	Stem + benzene
Bud + distilled	Bud + methanol	Bud + benzene

Extraction of plant: As we collected, grind with different solvents, dried at room temperature for 1day and take measure their weight, methanolic leaves(0.5gm), methanolic root (0.2gm), methanolic stem(0.4gm), methanolic bud (0.4gm). Distilled water leaves (0.4gm), distilled water root (0.3gm), distilled water stem (0.5gm), distilled water bud (0.3gm). Benzene leaves (0.5gm), Benzene root (0.2gm), Benzene stem (0.4gm), Benzene bud (0.2gm). Then add 2ml of distilled water in each solvent extraction and pour all the solvent extraction in 2ml of Eppendorf's tube and refrigerate it.

Collected and culture of larvae: Firstly, we collected larvae from the pit holes which was present behind the biotech department with the help of some bowl and then we cultured the larvae at room temperature by giving them food (3gm pedigree +1gm yeast). Then we took 13 white bowl and in each bowl pour 100ml filtered rain water and randomly we added 10 larvae of all the stage and the given food to each

bowl to minimise their stress condition after day 1 we added 1ml of all the extraction in each bowl. Next day we observed that only few larvae were affected in methanolic extraction and rest of the other solvent larvae were not affected. At same day again we pour 1ml of all the solvent extraction in each bowl as labelling, food and water too. Next day we again observed the bowl of larvae and observed that 50% population of larvae were affected only in methanolic extraction, rest of solvent extraction were not affected. And again, we added 1ml of different solvent in each bowl as per labelling, food and water too. Next day that we observed that 90% population of methanolic extraction were dead and rest of the other solvent extraction were not affected. Again, same day we provided food and water not solvent extraction in any of the bowl. Next day we observed that other than methanolic extraction, other solvent extraction was showing their effect like methanol bud, distilled water bud, benzene root and benzene bud. But at last, we came to the conclusion that methanolic extraction were more effective and effectively showing its effect on larvae 1st stage.

Effect of Blumea lacera effect on disc diffusion assay: The methanolic leaves extraction of plant Blumea lacera was effective against gram positive bacteria (Escherichia coli) it shows antibacterial effect at different concentration 4mm and 5mm (mm = millilitre) and shows zone of inhibition. And Blumea lacera methanolic leave plant extraction, it also shows its effect against fungi Trichophyton rubrum, which causes saviour skin disease, and also form zone of inhibition around it.

RESULT AND CONCLUSION

Table 2. Table of result.

Solvent / Days	**1st Day (1ml)**	**2nd Day (1ml)**	**3rd day**	**4th day**
Methanol + leaves	1st and 2nd stage affected	1st and 2nd stage affected	1st and 2nd stage affected	1st and 2nde stage affected
Methanol + stem	No effect	No effect	No effect	No effect
Methanol + root	No effect	No effect	No effect	No effect
Methanol + bud	No effect	No effect	1st and 2nd affected	No further development
Distilled + leaves	No e3ffect	No effect	No effect	No effect
Distilled + stem	No effect	No effect	No effect	No effect
Distilled + bud	No effect	No effect	2nd stage affected	No further development
Benzene + leaves	No effect	No effect	No effect	No effect
Benzene + stem	No effect	No effect	No effect	No effect
Benzene + root	No effect	No effect	3rd stage affected	No further development
Benzene + bud	No effect	No effect	3rd and 4th affected	No further development

Table 3. Table of control.

Sample/Days	1st day	2nd day	3rd day	4th day
Control	Further development	Further development	Further development	Further development

We took all the extraction and pour in Petri dish and kept the Petri dish at room temperature of 24 hours. After 24 hours all the solvent got evaporated and then we added 2ml of distilled water and with the help of specula we extracted the distilled water extraction then pour in 2ml Eppendorf's tube and refrigerate it. Firstly, we collected larvae from the pit holes which was present behind the biotech department with the help of some bowl and then we cultured the larvae at room temperature by giving them food (3gm pedigree +1gm yeast). Then we took 13 white bowl and in each bowl pour 100ml filtered rain water and randomly we added 10 larvae of all the stage and the given food to each bowl to minimise their stress condition after day 1 we added 1ml of all the extraction in each bowl.

Next day we observed that only few larvae were affected in methanolic extraction and rest of the other solvent larvae were not affected. At same day again we pour 1ml of all the solvent extraction in each bowl as labelling, food and water too. Next day we again observed the bowl of larvae and observed that 50% population of larvae were affected only in methanolic extraction, rest of solvent extraction were not affected. And again, we added 1ml of different solvent in each bowl as per labelling, food and water too. Next day that we observed that 90% population of methanolic extraction were dead and rest of the other solvent extraction were not affected. Again, same day we provided food and water not solvent extraction in any of the bowl. Next day we observed that other than methanolic extraction, other solvent extraction was showing their effect like methanol bud, distilled water bud, benzene root and benzene bud. But at last, we came to the conclusion that methanolic extraction were more effective and effectively showing its effect on larvae 1st stage.

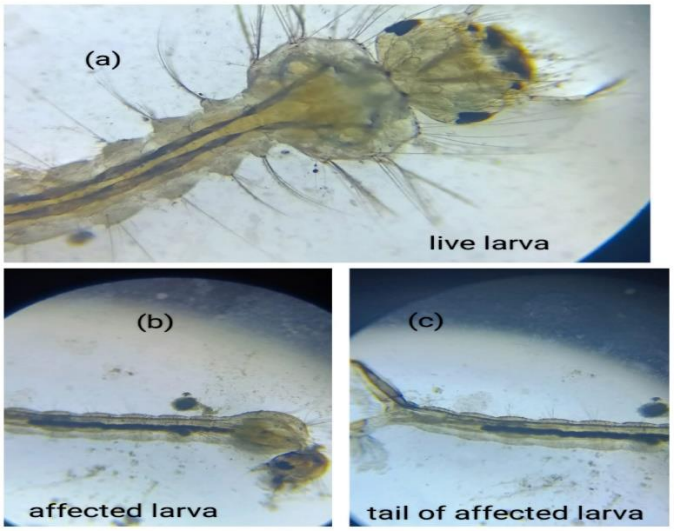

Figure 2. (a) Live larva) of Aedes aegypti in this figure narrow thorax is There, this shows that it is aedes aegypti, (b) Affected larva, (c) Tail of affected larva

Discussion: Plant based drugs and their chemical compound are being used to treat different disease and illnesses since the ancient time. Since the emergence of human folk fore, plants are being used considerably by all religious and culture for the betterment of human health and as a cure and treatment protocol for various illness [Sen et al., 2015]. As stated by World Health Organization (WHO), 80% world population dependent on plant-based medicine or ayurvedic to meet the principal health care. There are many plants whose secondary metabolites posses' properties like cytotoxic, antimicrobial, antifungal, antidiarrheal, antidepressants, anxiolytics, larvicidal, and antiulcer properties [Kaurinovic et al., 2013]. Antioxidants are substance that can cause reactive oxygen species (ROS)scavenging. Many numbers of reports say that plant phenolics are most effect effective group of secondary metabolites that plat role as an antioxidant(primary) or free radical scavengers and phenolic and tannins are the compounds that are found in Blumea lacera plant extraction. The presence of phenolics compound i.e Flavonoids and tannins in Blumea lacera are accountable for the free radical to produce scavenging effects [Anderson et al.,2012]. While quantitative test of Blumea lacera compound, the total phenolic compound was found 39.55mg/g. Cancer is major health issue in today's time for human health both in developed and developing countries. And blumea lacera methanolic leaves extraction is also accountable for showing antitumorigenic activity in different stages of cancer growth and development of cancer following diverse mechanisms including apoptosis property in tumour cells. And the cytotoxic effect of BLP may occur due to the presence of alkaloids, steroids [Alam et al., 2021]. As we all know bacteria keep on showing pr developing genetic resistance against drugs.so, to fight against this biotechnology have developed the varieties of antibiotics against various number of spectra. And BL extraction showed various number of properties like antimicrobial, antifungal, larvicidal and antitumoral etc due to the presence of numerous phenolic compounds [Su et al., 2020].

As we know that in today's time diarrhoea is major and inevitable death adults as well as infants, developing and developed countries, it causes due to the peristaltic movement in small intestine which cause inflammatory effect in the mucosa of the intestine to prostaglandins secretion of the intestinal wall, and in Blumea lacera plant extraction posses' antidiarrheal effect due to the presence phytoconstituents like flavonoids, and tannins. And some other phytoconstituents like alkaloids, terpenoids, cardiacglycosies present in blumea lacera plant extraction also responsible for antidiarrheal activity in human being model [Satya et al., 2015]. This plant is more frequently in many local areas as ayurvedic medicine without knowing its scientific reason. And plant extraction of blumea lacera do not affect the beneficial bacterial colonies in stomach e.g., Escherichia coli [Satyal et al., 2015].

Conclusion

In the experiment, Blumea lacera plant extraction showed its efficacy, antibacterial properties against Escherichia coli, and antifungal properties against Trichophyton rubrum, which cause sever skin disease. And most effectively it showed its efficacy in larvicidal property against the 1st stage of Aedes aegypti larvae which cause severe disease i.e malaria it may killed the mosquits larvae due to the presence of phytoconstituents like steroids, flavonoids, tannins, that can kill mosquitoes' larvae with high mortality rates.

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