Phytochemical Screening and Antibacterial Properties of (Leaves) *Mallotus Philippienensis* L. (Mull.)Arg.

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Abstract

An ethno medicinal plant, *Mallotus philippienensis* (Lam.) Muell. Arg., var. *philippienensis* was analyzed for chemical composition and antibacterial activity.Mallotus *philippienensis* of the family Euphorbiaceae is a large succulent tree used for dying and in medicines. The leaves are considered as bitter, cooling and appetizer.cause, flatulence and constipation are used as fodder and various type of medicinal value. The present study is the photochemical screening.antibecterial antimicrobial activity of the leaf extrect.The leaf extracts with various solvent like ethanol.methanol.distil water. Were done. It showed the presences of Carbohydrate, Fats, and oil, Falconoid, Glycoside, Saponins, Tannins, and Steroid. The plant extracts also antibacterial activity against the *Escherichia coli*, *Salmonella typhi* organisms. The results are indicating ethanol are showing good result of zone of inhibhions.It was observed that high concentration of all chemical analogized as compared of to all solvents. The extract of leaves of this plant is being used for further analysis in rural population of subcontinent since many centuries. This experiment will help to highlight the importance of these valuable organic compound found in this plant.

KEYWORDS- Ethno medicinal plant, phytochemical testing, *Mallotus philippienensis,* Antibacterial activity.

Abbreviations -M.phili.-Mallotus *philippienensis*; E.Ext -Ethanol extraction; M.Ext.-Methanol extraction; D/W -Distil water; E.coli- *Escherichia coli*, S.typhi-*Salmonella typhi*.

Introduction

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors' an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [Shariff, Z.U. (2001).]. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [Chung PY, Chung LY, Ngeow YF et al., 2004- Nair R, et all, 2005]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [Essawi T, Srour M., 2000.].

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources

[Mothana, R.A., Lindequist, U., 2005.]Many of the plant products are important therapeutic agents, which are represented by the various phytochemical like alkaloids,glucosides,flavanoids,mucilages,enzymes,etc.Even now about 75%of human population depends on plant extracts and products as a tool pf traditional medicine so human interest is concentrated on biodegradable and nature friendly products.

The Euphorbiaceae is the fourth largest family of the angiosperms comprising over 300 genera and about 7500 species distributed widely in tropical Africa [Gill, L.S. 1988.]. The euphorbiaceous plants are shrubs, trees, herbs or rarely lianas [Pandey, B.P., (2006).]. The family provides food [Etukudo, I. 2003] and varied medicinal properties used in ethno botany [Vasishta, P.C 1974 - Kubmarawa, D.et all 2007]. For instance, ricin contained *in M. philippienensis* is a well known poisonous compound that elicits violent purgative action in man [Trease, G.F and Evans, W.C. 2002]. In the traditional system of medicines, euphorbiaceae plants are used to treat various microbial diseases such as diarrhea, dysentery, skin infections and gonorrhea [Ajibesin, K.K., ET all, 2008]. The effects of plant extract on bacteria have been studied by a very large number of researches in different parts of the world [Ates, D.a and Erdogrul, O.T (2003)]. There are several reports in the literature regarding the antibacterial activity of crude extracts prepared from plants [El-seedi, H.R et all 2002-] Parekh, L., Chanda, S., (2006).]. The present study is concentrated on *M. philippienensis*, belong to family Euphorbiaceae. The plants of the genus *Mallotus* are a rich source of biologically active compounds such as phloroglucinols, tannins, terpenoids, coumarins, benzopyrans, and chalcons (An T.Y *et al* 2003; Chung Y.-C *et al* 2002; Van Kiem *et al* 2004; Likhitwitayawuid K. *et al* 2005). *Mallotus*, or of individual chemical constituents isolated from these extracts, have been reported by several authors. *Mallotus philippienensis* Muell. (*Euphorbiaceae*), also known as kamala or kamopollaka, was used as a source of a yellow dye (kamala dye), and as an antioxidant for ghee and vegetable oils (Rao V.S et al 1947).

Mallotus philippienensis (Lam.) M.Arg.is a woody medium sized much branch tree. It is a commonly dye yielding plant locally known as kamala, mostly found in India subcontinent. (Jaya Sharma et all 2012) Whole parts of the plants are rich in secondary metabolites, which impart medicinal uses to the plant. The plants have found application in pharmaceuticals as it is one of the common plants used in Indian system of medicine. Various parts of the plant are used in the treatment of skin problem, bronchitis, antifungal, tape worm, eye-disease, cancer, diabetes, diarrhea, jaundice, malaria, urinogenital infection etc. (Jaya Sharma et all 2011) In dispersing swellings of the joints from acute rheumatism and of the testes from suppressed gonorrhea. It also shows anti-oxidant, anti-bacterial, anti-fungal, anti-microbial insectidal /pesticide, anti-microfilaria, anti-lithic, heptoprotctive activities. Antimicrobial activity (Jayaraman velanganni *et al*., 2011).Antibacterial evaluation (K. Moorthy *et al* 2005).The leaves are considered Bitter, cooling and appetizer, cause flatulence,antifungal,antibacterial,anthelmintic,antioxidant activity and constipation, her are used as fodder. Thus this study aims at determining the antibacterial effects of this Euphorbiaceae plant.

Materials and Methods

The research study was conducted at the Department of Botany.Sarojini Naidu Govt... Girls Post Graduate (Autonomous) Collage Shivaji Nagar, Bhopal. Madhya Pradesh (India).

Sterilization of the equipments and disinfection

All the equipments were disinfected with cotton wool soaked in methylated spirit so as to maintain sterility throughout the process. Conical flasks and beaker test tubes and other glass were sterilized by hot air oven at 160 ºC for 45 minutes, whereas moisture insensitive materials were sterilized by autoclaving at 121ºC for15 minutes.

Source of the plan material, collection and authentication

The plant material of *Mallotus philippienensis were* collected in the month of March, 2011 from the tree growing inside the Botanical garden of BHEL College Bhopal. The plants were identified by Botanical survey of India, CRC (BSI) Allahabad, where voucher specimen code (1370-158-696) was deposited.

Preparation of Samples

After collection fresh plant materials were washed under running tap water, air dried, followed by oven drying. Finally the samples were crushed and converted into powdered form and stored in airtight bottles for further analysis.

Phytochemical analysis of Plant

The phytochemical screenings for analysis of secondary metabolites were followed. The phytochemical screening of the plant extract was carried out by following the method used by to detect the presence or absence of certain bioactive compounds. Different samples of this plant were analyzed for Alkaloid, Carbohydrate, Fats and oil, Flavonoid, Glycoside, Saponins, Tannins, and Steroid

Test Organism Used

The test microorganism like, *E.coli* and *S.taphi* were used. The microorganisms were collected from Department of Botany.Sarojini Naidu Govt. Girls Post Graduate (Autonomous) Collage Shivaji Nagar, Bhopal. Madhya Pradesh (India).

Experiment

***Methods of Extraction*:**

The fresh leaves were cleaned freeze-dried and grounded into fine powder using an electric blender. The powder was dried in an oven at 40°C for 24 h, then the fine powder was sieved through 24-mesh. The fine powdered sample (10g) was extracted with 250 ml 80% methanol in water at room temperature (.25°C) for 24 h in a shaking water bath. The extract was filtered by a Millipore filter with a 0.45µm nylon membrane under vacuum at 25°C. The samples were stored at 4°C until use. For aqueous extract the fine powdered sample (10g) was extracted with 100ml of distilled water.

Phytochemical screening

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans WC (2002).The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Carbohydrate

Treat the test solution with few drops of alcoholic alpha napthol. Add 0.2ml of con. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

Fats and oil

Add a few drops of 0.5N of alcoholic potassium hydroxide to small quantities of various extracts along with a drop of Phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of Fixed oils and Fats.

Flavonoid

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 - 6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and Orange color for flavones (Siddiqui and Ali, 1997).

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer (Siddiqui and Ali, 1997).

Saponins

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells is used as screening test for these compounds.

Tannins

To 0.5 ml of extract solution 1 ml of water and 1 - 2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins.

Steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids (Siddiqui and Ali, 1997).

Determination of Antibacterial Activity:

An agar-well diffusion method was employed for determination of antibacterial activities [11]. The stored leaves extract samples were dissolved in phosphate buffered saline (PBS, P 7.0-7.2). All bacteria were suspended in sterile water and diluted to10 CFU/ml. The suspension (100µL) was spread the surface of nutrient agar medium. Wells (4.6mm in diameter) were cut from the agar with a sterile borer and 60µL extract solutions were added into them. Negative controls were prepared using PBS solution. Penicillin G and gentamycin were used as positive reference standards to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 37°C for 24 h. diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicates.

Results

The qualitative phytochemical investigation of *M. philippienensis lem* L. Leaves extracts showed the presence of only Carbohydrate Flavanoids, *Glycoside*, Saponins, Tannins, and Steroids in all extracts.The M.ext. Of plant showed the presence of higher concentration of chemicals. Study showed that the all extracts of plant contains of phytochemical such as presence but E.ext are minimumD/W are normal and M.ext are showing very higher concentration of chemicals (Tabel. 1).*M. philippienensis* showed good activity against the bacterial strains *Escherichia coli, Salmonella taphi.* The antibacterial assay revealed that E.ext, of *M.philippenesis* possess very good zone of inhibition where as M.Ext. Having antibacterial activity only on higher concentration. (Table 2, 3, 4)

Table1.

Qualitative tests of leaf extract of *Mallotus philippienensis (*LAM.) M.Arg.In

Qualitative test	E. Extract	M. Extract	D/W Extract
Alkaloids	-	-	-
Carbohydrate	+	++	+
Fats and oils	-	-	-
Flavanoids	++	++	++
Glycoside	+	+++	++
Saponins	-	+	+
Tannins	+	+++	+
Steroids	-	-	+

Ethanol, Methanol and Distil water.

- = (negative result), + = (small amount), ++ = (average), +++ = (high)

Graph-: 1 qualitative test leaves of *Mallotus philippienensis* (LAM).M.Arg. In Ethanol, Methanol, and Distil water

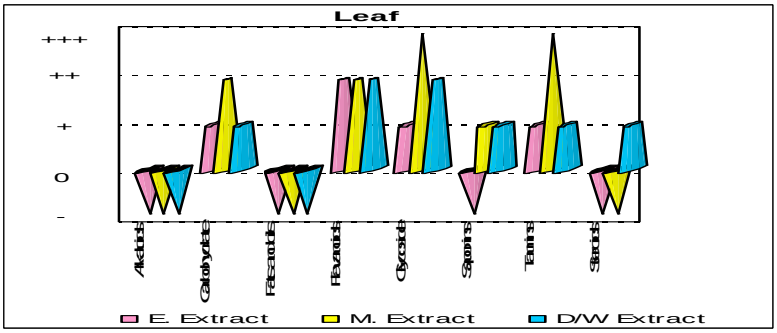


Table 2.

Antibacterial effect of leaf extracts of *M. philippienensis* L. of Concentration 50mg/ml

Test Organism		Diameter of Zone of Inhibition(mm) Extract of Concentration(50 mg/ml)		Standard Referend(mg/ml)	
	E.Ext	M.Ext.	D/W Ext.	Streptomycin	Chlormphenicol
E. coli	7.86±0.193	16.55±0.326	0	29.812±1.27	15.822±1.90
S. typhi	21.24±0.800	0	0	47.452±1.67	23.055±2.76

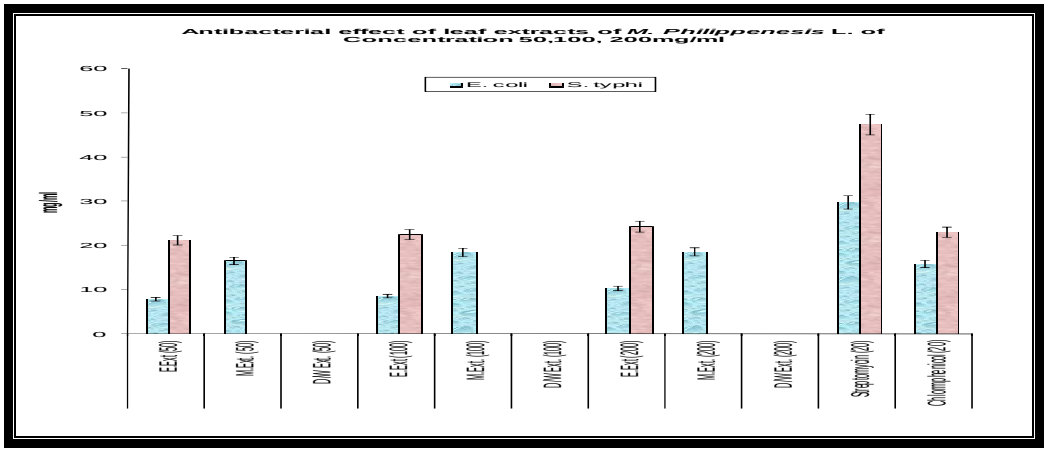
Table 3.

Antibacterial effect of leaf extracts of *M. philippienensis* L. of Concentration 100mg/ml

Test Organism		Diameter of Zone of Inhibition (mm) Extract of Concentration(100 mg/ml)		Standard Referend(mg/ml)	
	E.Ext	M.Ext	D/W Ext.	Streptomycin	Chlormphenicol
E. coli	8.56±0.274	18.46±0.464	0	29.812±1.27	15.822±1.90
S. typhi	22.47±0.960	0	0	47.452±1.67	23.055±2.76

Table 4.
Antibacterial effect of leaf extracts of *M. philippienensis* L. of Concentration 200mg/ml

Test Organism		Diameter of Zone of Inhibition (mm) Extract of Concentration(200 mg/ml)		Standard Referend(mg/ml)	
	E.Ext	**M.Ext**	**D/W Ext.**	**Streptomycin**	**Chlormphenicol**
E. coli	10.30±0.354	18.61±1.420	0	29.812±1.27	15.822±1.90
S. typhi	24.35±0.516	0	0	47.452±1.67	23.055±2.76



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Discussion

Phytochemical compounds of the plant were qualitavely analyzed. In the analysis of phytochemical compounds of *M. philippienensis* extract showed the presence of Alkaloids, Carbohydrate, Fats and oils, Flavonoids, Glycosides, Saponins, Tanins, and Steroids.(table 1.)The antibecrial studies were also conducted with ethanol, methnol, distil water extract of plant and showed wide variation (2, 3, 4).The antibacterial activity of these plant extract range form 7-24mm against the organism. The antibacterial of these plant forms the present the investigation. It was proved that the ethanol plant extract is efficient to control bacterial concentration of 200/mg/ml when compared to the standard Streptomycin against E, coli. And S. taphi (Table 2. 3, 4).

Currently the plant is facing a threat of extinction due to destructive of plant parts for medicinal use as well as devastation of its natural habitat by deforestation (Jaya Sharma ET all 2012).

The extracts were testing from leaves of this plant are used in traditional medicinal for curing various types of aliments. The research is main point of view compare to all part chemical concentration and I observed to leaves are showing to high value of chemicals. The Phytochemical components of the *M. philippienensis* have been established in previous studies and these include tannins, saponins, alkaloids, carbohydrates, phenols, flavonoids, sterols and resins [Zafar, R.et al.,1993].

These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some give plants their odours and or flavours and some still are responsible for their pigments. (V.Mary kensa* ET all 2011**)**

The results of antibacterial activity (Table 2,3,4) of the ethanol, methanol, and distil water leaf extract showed concentration dependent activity against all the tested bacteria with the zone of inhibition ranged from **7-24mm** at various concentrations. The solvents used for extraction were used as control and all the solvent control did not show any activity. Standard antibiotics were also used along with the extracts for comparison as given in the Table 2, 3, 4. (Measurement indicates the zone of inhibition in mm); Ethanol extract showed the maximum zone of inhibition ranged from 7.86+0.193 to 10.30+0.354 against bacteria such as 21.24+0.800 to24.35+0.516 26mm against at .50mg/ml- 200mg/ml concentration.

Phytochemical screening of the ethanol.methanol,distil water extracts of leaf of *Mallotus philippienensis* var. *philippienensis s* showed the presence of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins. The presence of secondary metabolites in plants produces some biological activity in man and animals and is responsible for their use as herbs in ailments (Sofowora A 1986). The major compound diethyl phthalate is used medicinally for the preparation of 67 consumer formulations including bath preparations (oils, tablets, and salts), eye shadow, toilet waters, perfumes and other fragrance preparations, hair sprays, wave sets, nail polish and enamel removers, nail extenders, bath soaps, detergents, aftershave lotions, and skin care preparations(Anonymous1985-.. Kamrin M A) and also as a component in insecticide sprays, mosquito repellents and camphor substitute. (World Health Organization; 2003.).

From the results of antimicrobial activity, it was found that the methanol distils water and ethanol extracts exhibited maximum antimicrobial activity against the tested human pathogens. In our study, the maximum zone of inhibition against bacteria such as *E. coli*. May be due to the presence of secondary metabolites such as flavonoids, any groups and steroids as suggested by previous reports. (Kosalec I 2005- Pereira AP 2007-. Lauro Figueroa 2008).

Even in hospitals, majority of disinfectants such as phenols, Lysol, cresols used are belonging to phenolic groups. Thus resent findings of antibacterial activity against *E.coli* , *S. typhi*, revealed the medicinal potential value of methanol and ethanol extracts against abdominal pain, diarrhea, fever, nausea, septocaemia, urinary tract infections and vomiting, caused by *E. coli*, hospital-acquired wound infections,septicaemia and urinary tract infections by *E.coli*, typhoid fever by *S. typhi* and diarrheal infections.

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Conclusion

Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Further investigations are required in order to isolate more new compounds from the plant extracts and to test

their bioactivities with the aim of increasing the drug arsenal currently used in the treatment and prophylaxis of human and animal diseases. This plant can be further subjected to isolation of the therapeutic antibacterial and carryout further pharmacological evaluation.

This investigation has opened up the possibility of the use of this plant in drug development for human consumption possible for the treatment of gastrointestinal, urinary tract and wound infections and typhoid fever. However, before coming to conclusive statement, further research is needed to investigate the antibacterial ingredients.

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