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PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VITRO ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF LEAF AND STEM OF *EUPHORBIA AMPLIPHYLLA PAX* (FAMILY-EUPHORBIACEAE)

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ABSTRACT

Folk medicines play an important role in the rural sectors of developing countries. Many plants based drugs of modern medicine are derived from traditional medicines. Most phytochemicals are secondary metabolites and are nonessential nutrients and most of them are produced as defensive. The majority of these phytochemicals are potential therapeutic agents. The present study is planned to reveal the phytochemical content and antibacterial activity of the succulent tree, *Euphorbia ampliphylla* of the Family Euphorbiaceae. Phytochemical analysis of the bark and leaf extract shows that it contains alkaloids and flavonoids. *Euphorbia ampliphylla* demonstrates significant antibacterial activity against gram-positive cocci and gram-negative bacilli.

KEYWORDS: Antibacterial activity, *Euphorbia ampliphylla*, phytochemicals, secondary metabolites, folk medicines.

INTRODUCTION

Plant based folk medicines have immense therapeutic potential against many diseases. The plant based drugs can contribute significant role in the treatment of emerging diseases. Plants protect themselves by producing different phytochemicals for their existence. Plants produce polyphenols like alkaloids and flavonoids which are proved as potential drugs. The health effects of polyphenols are immensely studied and witnessed by several publications.[1,2,3] Alkaloids, saponins, tannins, flavonoids and phenolic compounds are active secondary metabolites and are toxic to bacterial cells. Many of these products are free radical scavengers and are potent anticancer drugs. Regular consumption of polyphenols improves

immunity. Poly phenols are aromatic compounds derived from plants like caffeic acid, gallic acid, and common phenolic acid. Flavonoids are polyphenols with two aromatic rings linked together by three carbon atoms that form an oxygenated heterocycle. They are antioxidants, anti-inflammatory and antibacterial.[4] Triterpenoids are with six isoprene units and derived from squalene by the biosynthetic process. They are anticancerous, anti-inflammatory, antiproliferative and hepatoprotective.[5] Alkaloids are nitrogenous aromatic organic molecules. They are present in animals, plants, and mushrooms. Alkaloids are anticancerous, antimalarial, antiasthmatic and antibacterial. Alkaloids like atropine and tubocurarine are toxic.[6,7] Tannins are water-soluble polyphenolic biomolecules. They are anticancerous and antimutagenic.[8] Anthraquinones are a potential laxative, antioxidant, antibacterial, antifungal, antiviral and insecticidal. Continuous use of anthraquinones may cause melanosis coli.[9]

Many of the plant derived drugs have made large contributions to the well being of cattle and humans. A recent review revealed that about 119 plant-derived compounds are considered as important drugs in many countries. Of these around 77% are derived based on the knowledge attributed to traditional medicine. The rich biodiversity of the tropical countries represents about 250,000 to 500,000 species of plants. Of these only 1 to 10% is used as foods by humans and animals.[10,11] Family Euphorbiaceae is a large family consisting of about 7,000 species of plants varying from small herbs to large trees. Many of the researches revealed the medicinal importance of the family. *Euphorbia hirta*, commonly known as asthma weed and is used for the treatment of many diseases including arthritis inflammation.[12] *Euphorbia ampliphylla* is a succulent tree of the montane ecosystem widely distributed in Eritrea, Ethiopia, Somalia, Kenya, Uganda, Malawi and Zambia. The local name in Ethiopia is *qulqulae*. The latex of the plant is an eye irritant. It is widely used as a medicine in Kenya and Tanzania. The plant decoction is mixed with chicken soup or mutton soup in minor quantities and is used as a medicine for severe constipation, stomach ache and to expel placenta during childbirth. In some parts of Africa, a decoction is used as a traditional medicine for HIV and for treating wounds and sores of cattle.[1,4] It is noticed that scientific evaluation of phytochemicals, antioxidant activity or antibacterial activities of this plant is so far not done.

MATERIAL AND METHODS

Chemicals and reagents

Methanol, acetone, chloroform, petroleum ether, ethyl acetate, sulphuric acid, hydrochloric acid, glacial acetic acid, ammonia solution, potassium hydroxide, vanillin, benzene, lead acetate, mercuric chloride, and gentamycin. The reagents used were analytical grade and were purchased from sigma.

Collection and Identification of plant material

The plant Euphorbia ampliphylla was collected from Yebu, located at an altitude of about 1900 meters above the sea level. The plant was identified by Dr. Remesh, Dept of Biology, Jimma University and kept in the herbarium (voucher no.MS 40/2012). The collected plant material was cleaned and dried in the shade. Bark and leaves of the dried plant were grinded using electrical appliances and stored in airtight glass containers for further studies.

Preparation of the extract

18gms of powdered bark and leaves of *Euphorbia ampliphylla* were extracted with chloroform, acetone, and 80% methanol using Soxhlet apparatus. Each fraction was collected separately and the solvent was removed by evaporating in a rotary evaporator. The resultant extract was then quantified using a digital weighing machine and the percentage yield obtained from different solvent extractions was calculated.[15]

Phytochemical screening

The phytochemicals present in the plant extract were detected by using the method of Tura et al[22] and Dawo.[23]

Antibacterial activity of the bark and leaf

Antibacterial activities were studied using Muller Hinten Agar MHA (lot no.X4225E, Oxoid, England) and Nutrient broth (DEFCO laboratories the USA). Preparation and sterilization of the media were done using appropriate techniques according to manufactures guidelines. The bacterial strains were prepared by subculturing from standard strains obtained from the Microbiology Department, Jimma University. The bacterial strains used were *Staphylococcus aureus* (ATCC25925), *Escherichia coli* (ATCC25922), *Salmonella typhi* (ATCC-83859) and *Pseudomonas aeruginosa* (ATCC27853).[26]

The antibacterial activity of the bark and leaf extracts against selected bacterial strains was studied using the agar well diffusion method. All bacterial stock cultures were first subcultured on to blood agar plates and incubated at 37^{o}C for 24 hrs. From these subcultures, with a sterile bacterial loop, bacterial strains were inoculated into 5 ml nutrient broth and incubated overnight at 37^{o}C. OD of the subculture was measured using a spectrophotometer with 625nm wavelength and the absorbance was adjusted between 0.08 and 0.1. It is assumed that the adjusted suspension should contain about 12X107CFU of bacterial strains per ml.[17] The inoculums of the respective bacterial strains were streaked into Muller Hinton agar plates using a sterile cotton swab covering the entire portion of the culture plate ensuring even distribution of the organism. Separate plates were prepared for each strain. The dried mass obtained after the extraction was taken and a 12.7 mgm/ml stock solution was prepared by dissolving in DMSO. From this different concentration of the extract was prepared and tested against standard bacterial strains for antibacterial activity studies. Wells of 6mm diameter was formed on to the agar plates by using a sterile cork borer. All the extract of different concentrations was carefully inoculated into the well. Respective solvents (chloroform, acetone, and methanol) and a commercial disc of gentamycin (0.1mgm/ml) were used as a negative and positive control. Petridishes were then incubated at 37^{0}C for 24hrs. Finally, the media were checked for the zone of inhibition, measured and recorded in mm.[17,18]

Determination of minimum inhibitory concentration (MIC)

Muller Hinton agar plates were prepared according to manufactures directions and sterilized in an autoclave. To the 18ml of the molten media 2ml of the extract was incorporated and poured into the Petri dishes. Like these different concentrations of extracts incorporated culture media were prepared and properly labeled and kept in the refrigerator. These culture media were inoculated with different standard strains and the highest concentration and lowest dilution which inhibited the growth of the respective strains were identified and is recorded as MIC.[16]

RESULT

The ground plant materials were subjected to extraction procedures with different solvents like methanol, acetone, and chloroform. Table 1 reveals the phytochemical contents of the different solvent extracts of bark and leaves of the *Euphorbia ampliphylla*. It is noticed that methanol is the best solvent of choice compared to other solvents used. The studied plant has a high concentration of alkaloids, tannins, anthraquinones, triterpenoids, polyphenols, and

flavonoids. The result shows that both bark and leaves have almost the same phytochemical contents but triterpenoids and anthraquinones are in high concentration in the bark of the studied plant.

The antibacterial study shows that this plant has antibacterial property against gram-positive and gram-negative bacilli. The methanol extract of both bark and stem is more potent compared to the other studied solvents and also noticed that *Euphorbia ampliphylla* extract is resistant to *Salmonella typhi*. Even very tiny concentration (50µg/ml) of the solvent extract has a high potency of antibacterial activity. Chloroform extract is less potent against different bacterial strains compared to other studied solvent extracts.

The minimum inhibitory concentration studied indicates that 25µg/ml of the methanol extract was inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa* and 50µg/ml is the minimum inhibitory concentration against *Escherichia coli.*

Table 1: Preliminary Phytochemical Screening of Bark and Leaf Extracts of E. Ampliphylla.

Phytochemical constituents	Bark	Solvent used					
		Leaf					
	Methanol	Acetone	Chloroform	Methanol	Acetone	Chloroform
Alkaloids	+++	+	-	+++	+	-
Saponins	-	-	-	-	-	-
Tannins	+++	+	++	+++	+	+
Anthroquinones	+++	+	+	++	+	+
Glycosides	++	+	-	++	+	-
Polyphenols	+++	+++	+	+++	+++	+
Triterpenoids	+++	+	+++	++	+	+
Flavenoids	+++	+++	+++	+++	++	++
Phytosterols	++	++	-	++	+	-

Table 2: Antibacterial activities of different fraction of *E.ampliphylla* against selected strains of bacteria.

Solvent used	Conc. µg/ml	Bacterial strain and zone of inhibition in mm							
		Bark extract stock (12.7mg/ml)				Leaf extract stock (12.7mg/ml)			
		S.aureus	P.aerug.	E.coli	S.typhi	S.aureus	P.aerug.	E.coli	S.typh
Methanol	25	8.7+0.2	7.9+0.5	6.0+0.5	00	6.5+0.4	6.1+0.5	3.0+0.4	00
	50	14.3+0.3	13.1+0.6	12.4+0.6	00	13.4+0.5	12.3+0.5	11.0+0.6	00
	75	16.8+0.4	14.6+0.6	14.6+0.4	5.8+0.6	14.8+0.4	13.8+0.6	12.1+0.6	00
	100	18.1+0.3	16.8+0.5	15.8+0.5	6.7+0.6	16.2+0.6	14.0+0.4	12.8+0.3	00
acetone	25	8.1+0.3	9.2+0.4	7.2+0.6	00	6.0+0.6	7.2+0.5	4.4+0.5	00
	50	12.4+0.4	21.4+0.5	12.9+0.5	00	12.4+0.5	11.8+0.5	12.6+0.5	00
	75	14.7+0.3	13.6+0.6	13.9+0.3	00	13.8+0.6	12.6+0.6	11.8+0.6	00
	100	15.6+0.3	15.3+0.5	15.5+0.6	00	14.7+0.6	13.8+0.7	14.9+0.4	00
chloroform	25	6.2+0.4	5.1+0.6	4.9+0.5	00	4.5+0.5	4.3+0.4	3.1+0.6	00
	50	10.8+0.3	12.9+0.5	11.0+0.4	00	12.8+0.5	11.8+0.4	10.3+0.5	00
	75	14.3+0.4	13.6+0.5	11.8+0.5	00	13.4+0.6	12.8+0.5	11.0+0.5	00
	100	15.8+0.3	14.8+0.6	12.5+0.5	00	14.0+0.4	13.5+0.5	11.8+0.4	00
Gentamycin	0.1mg/ml	21.3+0.3	20.0+0.2	17.3+0.4	17.9+0.3	21.5+0.3	19.6+0.4	17.5+0.3	18.2+0.3
DMSO		00	00	00	00	00	00	00	00

*00- indicates bacterial growth. Values are mean zone of inhibition± standard deviation

Table 3: MIC of different solvent extracts of E.ampliphilla on bacterial strains.

Solvent used	Conc. µg/ml	bark				leaf			
		S.aureus	P.aeruginosa	E.coli	S.typhi	S.aureus	P.aeruginosa	E.coli	S.typhi
Methanol	100	+	+	+	+	+	+	+	-
	50	+	+	+	-	+	+	+	-
	25	+	+	+	-	+	+	-	-
	12.5	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-
Acetone	100	+	+	+	-	+	+	+	-
	50	+	+	+	-	+	+	+	-
	25	+	+	-	-	+	+	-	-
	12.5	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-
chloroform	100	+	+	+	-	+	+	+	-
	50	+	+	+	-	+	+	-	-
	25	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-
gentamycin	0.1mg/ml	+++	+++	+++	+++	+++	+++	+++	+++
DMSO		-	-	-	-	-	-	-	-

NB (+) indicates inhibition of bacterial growth, (-) indicates bacterial growth

DISCUSSION

The therapeutic usage of a plant depends on various phytochemicals present in it. These secondary metabolites are widely used in the treatment of various veterinary, poultry, and human diseases. The alkaloids have some protective role in plants. This capability of alkaloids is made use in the production of various medicines. Tannins are antifungal agents.

The ability of the flavonoids as a free radical scavenging substance is well known and this property is used to suppress cancer tumors and to prevent carcinogenesis.[4] Tannins are effective antibacterial agents and this property is based on their ability to arrest translation in prokaryotes.[8] A wide range of triterpenoids is present in plants. Of this, large number exhibit cytotoxicity and this property are made use in the treatment of breast cancer. Many triterpenoids exhibit anti-inflammatory and anticarcinogenic properties.[2]

The family Euphorbiae is a family of wide varieties of succulent herbs, shrubs, and trees. The plant can be easily identified by its toxic and highly irritant milky latex. Many plants of the family Euphorbiaceae show the antibacterial properties against gram-positive and gram-negative bacilli because of the presence of different phytochemicals.[18,19,21] This study on *Euphorbia ampliphylla* also exhibits high potency of antibacterial activity like other species of Euphorbia. Many valuable medicinal products have been producing from this family against different strains of bacteria and fungus, anticancerous substances and ayurvedic oils and creams for pain and inflammation.[20] Tribals of Kenya, Tanzania, and Ethiopia are using this plant as a traditional source to cure different ailments. These traditional therapeutic properties of *Euphorbia ampliphylla* maybe because of the useful phytochemicals present in it. In Kenya and Tanzania, this plant decoction is used to relieve constipation. This property might be due to the presence of anthraquinones present in it.[9]

The gel diffusion method was used to evaluate the antibacterial properties of different solvent extracts of Euphorbia ampliphylla against different strains of bacteria. The results indicated that methanol and acetone extract of both bark and leaf has the highest potency as an antibacterial substance against both gram-positive and gram-negative bacilli. This may be because of different phytochemicals like polyphenols, flavonoids, and triterpenoids.[4,5,8] The methanol extract of bark and leaf exhibited a high zone of inhibition against all the tested bacterial strains except *Salmonella typhi*. This is because of the efficacy of methanol as best solvents for the extraction of many phytochemicals including polyphenols and flavonoids.[24,25] Methanol and acetone extract of both bark and leaves displayed MIC of 25µg/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration of chloroform extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was 50µg/ml and that of *Escherichia coli* was 100µg/ml. The minimum inhibitory concentration of methanol extract of bark against *Escherichia coli* was 25µg/ml and that of leaf was50µg/ml. Acetone extracts of both bark and leaves displayed a

MIC of 50µg/ml. The minimum concentration of Chloroform extract against *Escherichia coli* was 50µg/ml for bark and 100µg/ml for leaves. The methanol extract of bark shows a very weak antibacterial activity against *Salmonella typhi* with a zone of inhibition 6.7mm and MIC was 100µg/ml. This study correlated with many international studies.[19,20,24] The results of this study reveal folkloric usage of *Euphorbia ampliphylla* and recommend that the studied plant may be used for developing suitable drugs of choice after conducting the pharmacological and toxicological evaluation.

CONCLUSION

This study emphasizes the relevance of *Euphorbia ampliphylla* as a folk medicine. Most of the solvent extracts of this plant displayed an inhibitory effect against selected strains of bacteria. The gradient extracts also reveal the dose-dependent antibacterial activity. Correlating to other studies, it can be concluded that the presence of a high concentration of polyphenols, flavonoids, triterpenoids, and other active ingredients and the antibacterial property, in future this plant may be useful in primary health care.

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