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Preliminary phytochemical screening and antimicrobial activities of various fractions of *Mallotus philippensis* Muell.

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The phytochemical studies and biochemical screening of various fractions, that is, hexane, chloroform, ethyl acetate, butanol and aqueous (F_1 to F_5) obtained from the medicinal plant *Mallotus phillipensis* were studied. The presence of alkaloids, flavonoids, glycosides, phenols, quinines, saponins, tannins and terpenoids were confirmed by performing chemical tests using standard protocols. The infrared (IR) spectroscopic analysis revealed the presence of oxygenated and carbo-nitro functionalities, mainly in the polar fractions of the plant. All the fractions were tested for their antimicrobial activities against nine of the human pathogens including *Bacillus subtilis, Staphylococcus aureus, Staphylococcus pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Aspergillus flavus, Aspergillus niger* and *Candida albicans* using standard procedures. Among all, ethyl acetate (F_3) and butanol (F_4) fraction exhibited strong antibacterial and antifungal activities. F_3 revealed to be the most promising by showing 80% inhibition zone in case of *P. vulgaris* (80%), *S. typhi* (80%), while almost 70% against *B. subtilis* (68%) and *S. pneumonia* (67%). In fungicidal assay, F_3 showed 75 and 73% inhibition against *A. flavus* and *A. niger*, respectively.

Key words: *Mallotus phillipensis*, phytochemical screening, infrared (IR) spectral analysis, antimicrobial activities.

INTRODUCTION

The genus *Mallotus* belongs to the family Euphorbiaceae which plays a major role in the ethno pharmacology of several areas of the world where they are indigenous. Plants of this genus has been used in folk medicine such as topical antiseptic, anthelminthic and useful in treatment of bronchitis, abdominal diseases, spleen enlargement as well as to treat chronic hepatitis in traditional Vietnamese medicine (Chi et al., 1997; Loi et al., 2001). The genus as a whole is significantly efficacious against helminth parasites infections (Singh et al., 1997; Khunkitti et al., 2000; Asha et al., 2001), especially its fruit used to cure worm constipation, infestation as well as abdominal diseases (Pandey et al., 1991). *Mallotus phillipensis*, locally known as *Kamala,* is a woody plant of this genus, having wide geographical range extending from North America, East Asia to Northern Indo-Pak. Medicinally, the bark juice of this plant is used in diarrhea and dysentery (Samy et al., 1998). The fruits and roots are strong laxative, anthelminthic, vulnerary, detergent, maturant, and

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carminative; while leaves of this plant were shown to strongly inhibit mouse skin tumor promotion (Reiko et al., 2008). Moreover, "Kamala oil, obtained from this plant is used as a constituent for tung oil (Tanaka et al., 1998). The diverse medicinal importance of genus *Mallotus* has prompted us to carry out investigation on *M. phillipensis*. This study reports the phytochemical screening and antimicrobial activities of *M. phillipensis*.

MATERIALS AND METHODS

Plant

The whole plant *M. phillipensis* was collected in June 2008 from village Kuwari, district Mansehra, Khyber Pakhtunkhwa, Pakistan. The plant was identified by Prof. Dr. Manzoor Ahmad, Botany Department, Government Post Graduate Collage Abbottabad, where a voucher specimen was deposited in the herbarium (Accession No. C-0027).

Extraction and isolation

The shed-dried powdered material of *M. phillipensis* (2.5 kg) was extracted with methanol (65 L) at room temperature for a period of seven days (3 × 65 L). The resulting extract was filtered and evaporated with the help of rotary evaporator to obtain greenish gummy crude (81.6 g). This methanolic crude was then successively partitioned into *n*-hexane (F_1, 14.2 g), chloroform (F_2, 14.8 g), ethyl acetate (F_3, 18.8 g), *n*-butanol (F_4, 14.1 g) and water soluble fractions (F_5, 15.7 g).

Phytochemical screening

Chemical tests were carried out on all the fractions (F_1 to F_5) of the *M. phillipensis* using standard procedures to identify the phyto-constituents as described by Sofowora (Pour et al., 2011), Trease (Paulraj et al., 2011) and Evans and Harborne (Rajan et al., 2011). The following are a brief description of the procedures.

Test for alkaloids

About 0.2 g of each extract was heated with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragondorff reagent were added. Orange red precipitate confirmed the presence of alkaloids.

Test for tannins

About 0.5 g of each extract was mixed with water and heated on water bath. It was filtered and few drops of ferric chloride were added to the filtrate. Dark green solution colour confirmed the presence of tannins.

Test for anthraquinones

About 0.5 g of each extract was boiled with 10% HCl for few minutes in water bath and filtered. Then it was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Then drops of 10% ammonia was added to the mixture and heated. Rose-pink color indicated the presence of anthraquinones.

Test for glycosides

All the fractions were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solutions A and B were added. Appearance of orange red precipitate indicated the presence of reducing sugars.

Test for saponins

About 0.2 g of each extract was shaken with 5 ml of distilled water. It was then heated to boil. Appearance of creamy miss of small bubbles (Frothing) confirmed the presence of saponin.

Test for flavonoids

About 0.2 g of each extract was dissolved in dilute NaOH. After it HCl was added to the mixture. A yellow solution that turns colorless confirmed the presence of flavonoids.

Test for phlobatanins

About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate confirmed the presence of phlobatanins.

Test for steroids

About 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H_2SO_4. The colour changed from violet to blue or green in some samples confirmed the presence of steroids.

Test for terpenoids (Salkowski's test)

About 0.2 g of each of the extract was mixed with 2 ml of chloroform ($CHCl_3$) and 3 ml of concentrated H_2SO_4 was carefully added from a layer. A reddish brown coloration of the interface was formed to indicate the positive results for the presence of terpenoids.

Test for reducing sugars (Fehling's test)

A small portion of each of the extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solutions A and B for 2 min. An orange-red precipitate on boiling with Fehling's solution indicated the presence of reducing sugars.

IR Spectroscopy

Perkin Elmer Spectrum 100FT was used for IR spectroscopic analysis. The fractions were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the Fourier transform-infrared (FTIR) and scanned through the IR region. The characteristic absorption peaks in IR spectra of all the fractions are shown in Table 2.

Antibacterial activity

The antibacterial activity was checked by the agar–well diffusion method (Kavanagh, 1963). In this method, one loop full of 24 h old culture containing approximately 104 to 106 CFU was spread on

Table 1. Preliminary phytochemical screening of the various fractions of *M. phillipensis*.

S/N	Class	F_1	F_2	F_3	F_4	F_5
01	Alkaloids	-	+	+	-	-
02	Tannins	-	-	++	-	-
03	Glycosides	-	+	+++	+	+
04	Anthraquinones	-	-	++	-	-
05	Reducing sugars	-	+	+	+	+++
06	Saponins	-	-	++	-	-
07	Flavonoids	+	++	+++	++	++
08	Phlobatanins	-	+	+	+	+
09	Steroids	-	+	+	+	+
10	Terpenoids	-	-	+++	+	+

- = Absent, + = Present, ++ = Present appreciable, +++ = Present very appreciable.

Table 2. IR Spectroscopic data of solvent extract of *M. philippensis* (absprotion in cm^{-1}).

Functionality	F_1	F_2	F_3	F_4	F_5
O - H	3296.35	3381.21	3365.78	3290.59	3323.35
C = O	1924.28	1726.21	1683.86	1732.08	1730.15
C = C	1604.77	1614.42	1608.63	1606.70	1611.45
C - H	2922.25	2927.06	2925.04	2927.02	2925.07
C - O	1186.73	1071.48	1108.28	1043.57	1065.78
C – N	1355.85	1345.77	1233.54	1345.98	1356.63

the surface of Mueller-Hinton agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solutions of the test samples (F_1 to F_4) in the concentration of 1 mg/ml were prepared in dimethyl sulfoxide (DMSO) and 100 µl dilutions were added in their respective wells. The antibacterial activities of all the fractions were compared with standard drug, streptomycin which served as positive control.

Antifungal activity

The antifungal activity was determined by the agar well diffusion method (Kavanagh, 1963). In this method, miconazole were used as the standard drug. All the fractions (F_1 to F_4) were dissolved in DMSO (50 mg/5 ml). Sterile Sabouraud's dextrose agar medium (5 ml) was placed in a test tube and inoculated with the sample solutions (400 µg/ml) kept in slanting position at room temperature overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29°C and growth inhibition was observed.

RESULTS

The results of different experiments performed for the phytochemical investigations revealed the presence of flavonoids in all fractions (F_1 to F_5). Glycosides, reducing sugars, phlobatanins and steroids were also present in all fractions except F_1. Terpenoids were present in F_3, F_4 and F_5, but absent in F_1 and F_2. The presence of alkaloids was confirmed in F_2 and F_3 only. Tannins, anthraquinones and saponins were found only in F_3 (Table 1).

The IR spectra of all the fractions (F_1 to F_5) were obtained to confirm the presence of various functionalities (Egwaikidi et al., 2009). The IR spectra exhibited characteristic absorption for OH group in the region between 3296.35 and 3381.21 cm^{-1}, the absorption appeared between 2927.06 to 2922.25 cm^{-1} due to C-H stretching indicating aliphatic groups, absorption in the region 1924.35 and 1683.86 cm^{-1} clearly indicated the presence of carbonyls in all the fractions. Absorption bands for the olefinic functionalities appeared in between 1604.77 and 1614.42 cm^{-1}, while the indication of carboxy functional groups was found at 1186.73 and 1043.57 cm^{-1}. The absorption bands at 1355.85, 1345.77, 1233.54, 1345.98 and 1356.63 cm^{-1} showed the presence of C-N bond stretching (Table 2).

DISCUSSION

Antimicrobial activity

Antibacterial activity of various fractions (F_1 to F_4) of *M. phillipensis* were performed against six human pathogens including *Bacillus subtilis, Staphylococcus aureus, Staphylococcus pneumonia, Pseudomonas aeruginosa,*

Table 3. Antimicrobial activities of fractions (F_1 to F_4) of *M. phillipensis*.

Fraction	Gram positive bacteria[a]			Gram negative bacteria			Fungi		
	BS	SA	SP	PA	PV	ST	AF	AN	CA
F_1	6	5	5	4	5	4	6	5	4
F_2	12	14	11	12	17	15	14	10	12
F_3	22	20	20	18	22	24	24	22	20
F_4	16	14	15	16	18	15	15	18	14
Positive control[b,c]	32	34	30	32	34	30	32	30	34

a = Zone of inhibition in mm, b = Streptomycin (Standard drug in antibacterial assay, mg/ml), c = Miconazole (Standard drug in antifungal assay, mg/ml). BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, SP = *Staphylococcus pneumonia*, PA = *Pseudomonas aeruginosa*, PV = *Proteus vulgaris*, ST = *Salmonella typhi*, AF = *Aspergillus flavus*, AN = *Aspergillus niger*, CA = *Candida albicans*.

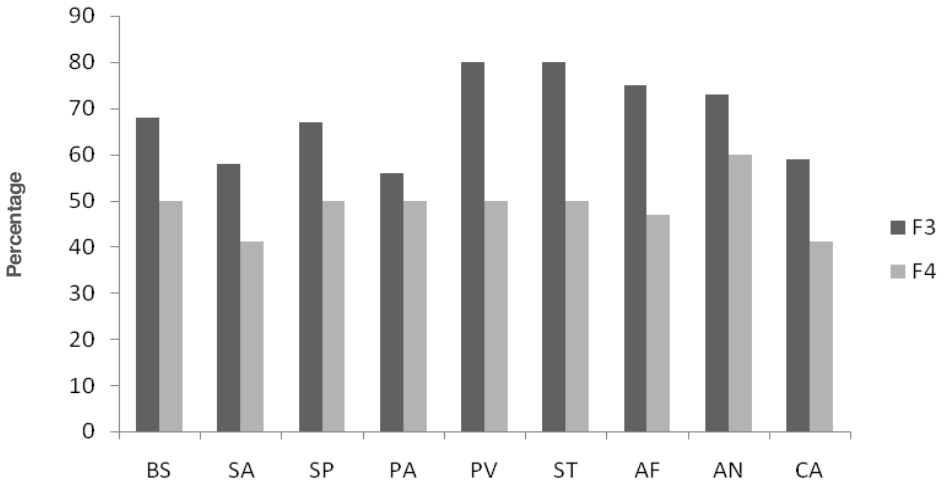

Figure 1. Percentage inhibition of fraction F_3 and F_4 in antimicrobial assays against all the tested organisms. BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, SP = *Staphylococcus pneumonia*, PA = *Pseudomonas aeruginosa*, PV = *Proteus vulgaris*, ST = *Salmonella typhi*, AF = *Aspergillus flavus*, AN = *Aspergillus niger*, CA = *Candida albicans*.

Proteus vulgaris, Salmonella typhi. The results are shown in Table 1. Fraction F_3 displayed the highest activity amongst all by inhibiting *P. vulgaris* (80%), *S. typhi* (80%), *B. subtilis* (68 %) and *S. pneumonia* (67%), while it showed minimum activity against *P. aeruginosa* (36%). F_4 revealed moderate inhibitory potential against all the tested cultures as it showed 50% of the inhibition zone against almost all the bacteria. F_2 showed moderate to weak inhibition, while F_1 revealed minimum activity against all the bacteria. These results were compared with standard drug (Streptomycin) which was more effective by showing maximum inhibition zones (Table 3).

Antifungal activity

The fungicidal activity of the various fractions (F_1 to F_4) of *M. phillipensis* was evaluated against three fungi including *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* (Table 1). The results indicated that both fraction F_3 and F_4 displayed significant activity while fraction F_1 and F_2 showed low activity in killing all the three tested fungi. The area of inhibition (in percent) for the F_3 was promising compared to the standard drug (Miconazole) against *A. flavus* (75%), *A. niger* (73%), while moderate inhibition was observed in case of *C. albicans* (59%). It was further observed that the fraction F_4 showed high activity against *A. niger* (60%), while weak activity against the rest of fungi. The fractions F_2 remained less effective, while F_1 showed no activity in killing the tested fungi (Figure 1).

Conclusion

Phytochemicals studies of *M. phillipensis* Muell. reveal the presences of several secondary metabolites, e.g. alkaloids, tannins, glycosides, saponins, flavonoids and terpenoids. The chemical tests as well as the IR spectral data were significant in identifying the presence of

various functionalities in the fractions. Furthermore, ethyl acetate and butanol soluble fractions (F_3 and F_4) showed maximum inhibition zone in antimicrobial assay, hence signifying the therapeutic effect which strongly supports the conventional use of this plant against various diseases. These activities may be due to the presence of biologically active compounds present as alkaloids and the glycosides (Cheng et al., 1998). The inhibition against fungi may be attributed towards potent monoterpenes, flavonoids (Kosalec et al., 2005; Pereira et al., 2007; Lauro et al., 2008) and steroids, which shows more activity in higher concentration against the growth of all fungi (Lauro et al., 2008; Winkelhausen et al., 2005; Subhisha et al., 2005). These results findings confirm the medicinal perspective of the ethyl acetate and butanol fractions against septicaemia, urinary tract and typhoid. It is further suggested that more phytochemical investigation should be carried out to isolate and characterize the potent compounds.

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