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ANTIMICROBIAL AND TOXICOLOGICAL STUDIES ON *MALLOTUS PHILIPPENSIS,* (KAMALA POWDER)

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ABSTRACT

Methanolic extract of Hairs and Glands covering fruits of *Mallotus philippinensis* (kamala powder) were subjected to Antimicrobial and toxicological screening. Antimicrobial profile indicated that extract was effective against all Gram positive cocci and bacilli used in the study. Among Gram negative bacilli, three important pathogenic species i.e. *Salmonella typhi*, *Salmonella para typhiA* and *Pseudomonas aeroginosa* showed sensitivity to the plant extract. Among Fungi tested, *Candida albicans* was sensitive whereas penicillium and Aspergillus species were non-sensitive to the extract. Acute toxicity studies in mice indicated that kamala powder is feebly toxic. No mortality is observed even at a high dose of 1000µm/kg B.W. However male mice exhibited significant decrease in body weight. Results of autopsy also showed significant decrease in weight of Liver and spleen at $P<0.05$ Histopathological studies indicated minor pathological changes in kidneys and liver of the test animals.

Keywords: *Mallotus philippensis,* Antimicrobial activity, toxicity.

INTRODUCTION

Mallotus philippensis Muell Arg (Kamala) is a shrub or a small ever green tree that is widely distributed in central, western, northern and southern Indo-Pak region. It is indigenous to south eastern Asia and found wild throughout tropical Asia, Australia and Philippines [1, 2]. It grows at a height of 4-10 meters, occasionally ascending to 1500 m, with the branchlets, young leaves and inflorescence covered with brown hairs. During the months of February-March its fruits ripen becoming black in colours. Mature fruits are collected and hairs and glands are gently separated from them [3]. The reddish brown powder thus obtained is used in traditional system of medicine as anthelmintic and vermifuge [4]. It is also used for treating moist condition of skin irritation, ringworm and other wounds. The powder is also used in skin and dandruff preparations and in Shampoos [5]. Other parts of the plant also have medicinal value, for example its leaves are reported to have anti-filarial activity [6], bark has antimicrobial properties [7] and fruits have anti-allergic properties [8]. Screening of literature revealed that only bark and leaves of the plant have been investigated with respect to antimicrobial activity [9-11]. Though that the drug kamala or kampillaka that is used in traditional system of Medicine refers to the red powdry glandular pubescence covering the ripe capsules (phalarajah). No report is available in literature on the antimicrobial or toxicity studies of kamala powder. This seems to be the first report on the evaluation of antimicrobial properties and toxicity of kamala powder.

MATERIALS AND METHODS

Plant material: Kamala powder was obtained from local market. It was authenticated at the department of Botany, University of Karachi and a voucher

specimen (86411) was deposited in the herbarium of same department.

***Extraction method of kamala*:** 50 gms of glands and hairs of fruit (Reddish brown powdery material) was soaked in 1500ml of methanol at room temp for 48 hours. The sample was filtered through whatman filter paper 1 to remove coarse particles and then suction filtered. The filtrate was evaporated to dryness under reduced pressure. This gave crude extract 7.3 gm (14.6% yield).

Experimental animals: Male and female NMRI mice weighing 20 – 30gms were used throughout the studies. The animals were bred in the animal house of HMI institute of Pharmacology and herbal sciences. They were housed in appropriate cages of Plexiglas material with 5-6 animals per cage. All the protocols of animal maintenance and handling were in accordance to internationally accepted guidelines. They were approved by institutional animal ethics committee.

ANTIMICROBIAL STUDIES

Microorganism used: The antimicrobial profile was investigated against thirty bacterial isolates (10 Gram +ve and 20 Gram–ve) and five fungal species. Gram positive isolates included *Staphylococcus aureus* (n=06), *Staphylococcus epidermidis* (n=02) and *Bacillus cereus* (n=02) where as Gram negative species included *Escherichia coli* (n=07), *Klebsiella pneumoniae* (n=05), *Proteus vulgaris* (n=02), *Pseudomonas aeroginosa* (n=02), *Salmonella typhi* (n=03) and *salmonella para A* (n=01). *Fungal isolates* comprised of *Candida albicans* (n=03), *Aspergillus species* (n=01) and *Penicillium Sp* (n=01). Bacterial cultures were kept at 4^{o}C on Mueller Hinton agar slants and transferred to fresh slants at monthly intervals. Fungal cultures were maintained on Sabouraud's dextrose agar. They were also kept at 4^{o}C and periodically transferred.

Preparation of Disc: The test compound was dissolved in methanol to make a stock solution of 1000µg /ml for further dilution. Sterile blank filter paper disc of 6mm diameter were impregnated with 20µg of the solution per disc.

The discs were left for nearly 30 minutes for complete absorbtion of the extract. The disc of negative control was prepared by using only methanol. Standard Augmentin disc (30µg) and standard Fungizone disc (25µg) were used as positive control in antibacterial and antifungal studies respectively.

***Determination of Antibacterial activity*:** The antibacterial efficacy of the methanolic extract of kamala was determined by disc diffusion technique of Bauer et al 1966. The extract impregnated disc and discs of negative and positive control were placed on Mueller Hinton agar plates that were seeded with 6-8 hour old culture grown in Mueller-Hinton broth. The turbidity of the culture was compared with that of 0.5 McFarland turbidity standards. The plates were incubated at 37^{o}C for 24 hours. After incubation, the zones of inhibition of growth around disc were measured to nearest millimeter. The mean of triplicate reading were used to interpret the results. The zones of inhibition of growth of 8mm or more were considered significant.

Determination of anti fungal activity: Fungal cultures were maintained on Sabouraud's dextrose agar slants at 4^{o}C. For the test, inoculum was prepared by swabbing the culture with sterile cotton swab and transferring it to a tube of Sabouraud's dextrose broth. The tubes were kept at 25-30^{0}C for 3-5 days. For assay, the plates of Sabouraud dextrose agar were seeded with fungal culture. The prepared disc of plant extract, disc of negative control and positive control Fungizone (25µg), were placed on the surface of agar. The plates were incubated at 25-30^{o}C for 5-7 days. The plates were checked after every 24hours for 5-10 days and results were recorded accordingly.

Toxicological studies: Acute oral toxicity of methanolic extract of kamala was determined in vivo in albino mice NMRI strain per oral route of administration via Oro-gastric tube. Two sets of animals were used. Animals of Group "T" were given the limit dose of 1000mg/kg body weight. Group "C" served as control, received equal volume of normal saline. Animals of both groups were kept under observation for three hours to note any change in general behaviour and Physiological activity.

All animals were weighed daily to note any change in body weight. The number of survivors was also noted daily. After ten days the surviving animals were subjected to autopsy. Heart, liver, spleen and kidneys were removed. The organs were blotted and weighed immediately.

Histopathological studies: Kidneys, heart, liver and spleen were fixed in 10% formalin solution. After usual process of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 5-µm slices through Leica RM2145 Rotation Microtome. The tissues were

stained with haexatoxylin and eosin. The slides were photographed using Nikon Trinocular research microscope OPTIPHOT equipped with NIKON photographic system and phase-contrast N-plan.

Statistical analysis: All data was analyzed using standard statistical tools. The comparison of average values of various parameters obtained from control and treated group was done by using student t-test. All results were considered significant when $P<0.05$.

RESULTS

Antimicrobial activity: Antimicrobial activity of the extract as determined by Bauer and Kirby disc diffusion method [12] exhibited interesting results. The methanol extract effectively inhibited growth of all Gram positive cocci and bacilli used in the study, with zones of inhibition ranging from 8mm to 14mm.

They also showed sensitivity to Augmentin (positive control) with zones of inhibition ranging from 8-15mm (Table 1). In case of Gram negative bacilli, strains of *Escherichia coli* and *Klebsiella pneumonia* did not show any inhibition of growth with test extract or Augmentin. However all the three strains of *Salmonella typhi (St1, St2, St3)* showed zone of inhibition of growth of 12mm, 12mm and 13mm respectively but they exhibited resistance to Augmentin. *Proteus vulgaris* was also non-sensitive to both test extract and Augmentin.

The result of sensitivity test of *Pseudomonas aeroginosa (Pa1, and Pa2)* indicated sensitivity of the organisms to test extract with zone of inhibition of 12-13 mm. The organisms were however resistant to Augmentin (Table 1).

The antifungal activity of the extract was determined with five strains of fungi. It was found effective against three strains of *Candida albicans* used in the study with zones of inhibition ranging from 10-12mm. These organisms also exhibited sensitivity against the positive control Fungizone 25µg with zone of inhibition varying from 14-16mm. However, the other fungi used in the study Penicillium Sp and Aspergillus Sp did not show susceptibility to the test extract or positive control fungizone (Table 2).

Acute toxicity studies: Acute toxicity studies were carried out in Albino mice NMRI (22 – 30gms). 10 mice (5 male + 5 female) were treated with 1000mg/kg body weight of the extract. The animals were kept under observation and checked after 1st, 2nd and 3rd hour for any change in behaviour. Dosing with test compound was continued for ten days. The mice showed high tolerance to test substance and no mortality was observed during the test period. However a significant decrease in the weight of the treated males was noted at $P<0.05$. In females the change of weight was non-significant (Table 3).

All animals included in the study were autopsied Heart, liver, kidneys and spleen and abdominal and pleural cavities were examined. They did not exhibit any gross change or abnormality. However treated organs showed significant decrease in weight of liver and spleen at $P<0.05$ and a non-significant decrease of weight in case of heart and kidneys (Table 4).

HISTOPATHOLOGY

Kidneys: The sections of kidneys revealed normal architecture of different regions such as glomeruli, tubules, Bowman's capsule and renal tubules. However focal areas of kidneys revealed mild chronic interstitial inflammation (Fig 1 & 2).

Liver: The section of liver exhibited preserve structure of hexagonal tubules, bile duct, hepatic artery and portal vein radicals. But central vein appeared dilated with hepatocytes showing pale wispy cyloplasm with reactive nuclear changes (Fig 3 & 4).

Heart: Sections of heart did not exhibit any remarkable change. (Fig 5 & 6).

Spleen: Remained unremarkable (Fig 7 & 8).

DISCUSSION

The results of the antimicrobial activity of the extract of kamala powder confirmed the folkloric use of the plant. As indicated in literature, *Mallotus phillippinensis (kamala powder)* is used commonly for the treatment of skin infection, surgical wound and wounds in which puss has been formed [5]. Such infection are usually caused by co-agulase positive *Staphylococcus aureus* that have been found sensitive to plant extract with zones of inhibition ranging from 8mm to 14mm (Table 1).

Very few reports on the antimicrobial activity of the plant are available in literature and that too are mostly related with extract of the bark of the plant.

Taylor et al 1996 indicated that extract of the bark of the plant was effective against strains of *Staphylococcus aureus* and *Bacillus subtilis*. However it did not exhibit any activity against four Fungi used in the study. In a similar study Mahato

and Chaudhry in 2005 reported that extract of the bark was active against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeroginosa*). Gangone-pieboji in 2009 indicated that extract of the leaves of *Mallotus philippinensis* was active against Gram positive cocci but not against Gram negative bacilli.

In 2010 Kadowaki reported [13] that extract of skin of the fruit of plant had high anti *Helicobacter pylori* activity. Present study seems to be first detailed report on the antimicrobial activity of kamala powder. The extract was effective against all Gram + cocci and bacilli used in the study. However Gram negative bacilli exhibited different pattern.

Three very important pathogenic Gram negative bacilli i.e. *Salmonella typhi, Salmonella para typhiA* and *Pseudomonas aeroginosa* showed sensitivity to kamala extract. All of them were resistant to Augmentin (30µg) a broad spectrum antibiotic commonly used for treatment of bacterial infection (Table 1).

Only one report in literature is cited on the antifungal activity of the extract of the bark of *Mallotus phillippinesis*. Taylor et al in 1966 indicated that extract from the bark of the plant was inactive against *Candida albicans, Aspergillus fungates, Trichophyton mentagrophytes* and *Saccharomyces cerevisiae.* In the present study the three strains of Candida albicans showed sensitivity to test extract. However Penicillium and Aspergillus species were non-sensitive to extract. The Literature on the toxicity of *Mallotus phillippinensis* is very scanty. The available report mainly deals with effect of extract of seeds on fertility of mice and rats that was reduced under the influence of test substance [14-16]. Current study is the first report on the toxicity of kamala powder. From the results it can be concluded that it is feebly toxic. The mice showed high tolerance to substance as no mortality is observed even at a high dose of 1000mg/kg. However male treated mice exhibited significant decrease in bodyweight. Results of autopsy indicated no apparent change in Kidneys, liver, heart and spleen except that Liver and spleen of the treated animal showed significant decrease in weight at $P<0.05$ (Table 4).

The histopathological studies indicated that in high dose 1000mg/kg body weight kamala powder produced minor pathological changes in kidneys and liver of the test animal (Fig 3, 4, 5, 6).

CONCLUSION

The data obtained from this study confirmed that kamala extract can be successfully used in the treatment of skin infections caused by co-agulase positive *Staphylococcus aureus*, as all the strains of *Staphylococcus aureus* included the study were sensitive to the extract.

The extract was also effective against *Salmonella typhi, Salmonella para typhi A* and *Pseudomonas aeroginosa,* three very important Gram negative pathogenic bacilli that are involved in numerous human ailments. It is pertinent to note that these organisms exhibited resistance to augmentin (30 µg) a known antibiotic used for the treatment of bacterial infection. Kamala can also be used for treating infections caused by *Candida albicans* as all the three strains used in the study were sensitive to Kamala extract. Acute toxicity studies in mice indicated that it is feebly toxic. Histopathological studies showed minor changes in kidneys and of test animals. From this study it can be concluded that kamala can be therapeutically used with careful management of the dose.

Table 1: Antibacterial activity of kamala extract against Gram positive (n=10) and Gram negative (n=20) bacteria

Code	Species Name	Diameter of zone of inhibition (mm)		
		Test extract	Augmentin 30µg	Negative control
Sa1	Staphylococcus aureus	8	15	-
Sa2	Staphylococcus aureus	14	12	-
Sa3	Staphylococcus aureus	8	15	-
Sa4	Staphylococcus aureus	10	10	-
Sa5	Staphylococcus aureus	8	12	-
Sa6	Staphylococcus aureus	12	12	-
Se1	Staphylococcus epidermidis	10	14	-
Se2	Staphylococcus epidermidis	14	12	-
Bc1	Bacillus cereus	9	8	-

Bc2	Bacillus cereus	10	8	-
Ec1	Escherichia coli	-	-	-
Ec2	Escherichia coli	-	-	-
Ec3	Escherichia coli	-	-	-
Ec4	Escherichia coli	-	-	-
Ec5	Escherichia coli	-	-	-
Ec6	Escherichia coli	-	-	-
Ec7	Escherichia coli	-	-	-
Kp1	Klebsiella pneumoniae	-	-	-
Kp2	Klebsiella pneumoniae	-	-	-
Kp3	Klebsiella pneumoniae	-	-	-
Kp4	Klebsiella pneumoniae	-	-	-
Kp5	Klebsiella pneumoniae	-	-	-
Pv1	Proteus vulgaris	-	-	-
Pv2	Proteus vulgaris	-	-	-
Pa1	Pseudomonas aeroginosa	12	-	-
Pa2	Pseudomonas aeroginosa	13	-	-
St1	Salmonella typhi	12	-	-
St2	Salmonella typhi	12	-	-
St3	Salmonella typhi	13	-	-
Sp1	Salmonella para typhi A	14	-	-

Table 2: Antifungal activity of kamala extract (n=5)

Code	Species Name	Diameter of zone of inhibition (mm)		
		Test extract	Fungizone 25µg	Negative control
Ca1	Candida albicans	10	14	-
Ca2	Candida albicans	12	16	-
Ca3	Candida albicans	12	14	-
As1	Aspergillus Species	-	-	-
Pe1	Penicillium Species	-	-	-

Table 3: Body weight of control and treated mice with Kamala extract

Mice	Control (gm)	Treated (gm)
Male	23.08 ± 2.318	20.53 ± 1.203
Female	20.70 ± 2.40	20.68 ± 2.169

n=6 for control and n=10 for treated

Table 4: Weight change of organs isolated from animals treated with Kamala extract

Organ	Control (gm)	Treated (gm)
Liver	1.0006 ± 0.4455	0.9879 ± 0.3168
Heart	0.1325 ± 0.0119	0.10957 ± 0.0226
Spleen	0.1411 ± 0.0340	0.11694 ± 0.0774
Right kidney	0.1712 ± 0.0346	0.12007 ± 0.0321
Left kidney	0.1498 ± 0.0205	0.19442 ± 0.02457

n=6 for control group and n=10 for treated group

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