ORIGINAL RESEARCH

Antibacterial Activity And Identification Of Phytochemical Components Of Leaf Extracts Of Mallots Philippensis

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ABSTRACT
The use of medicinal plants have been constant since the time of immemorial and the success of modern medical science largely depends on drugs originally obtained from natural resources, to such a degree that the World Health Organization (WHO) recognizes its important value. Kumkum is one of the medicinal plants from Garhwal Himalyan range was screened for the presence of major phytochemical compounds and also analyzed for their antioxidant activity. Klebsiella pneumoniae, a proteobacterian, an extremely resilient bacterium whose success as a pathogen seems to follow the model of "the best defense for a pathogen is a good defense" rather than "the best defense for a pathogen is a good offense". The 70% methanolic & ethanolic extracts of medicinal plants were prepared via, maceration process. Further, phytochemical analysis of each extract was considered & Kirby-Bauer Disk Diffusion Susceptibility Test was followed simultaneously. The results showed to be positive with maximum of the phytochemical tests i.e. alkaloids, carbohydrates, saponins, tannins, proteins, resin, phenols, later conducted disc diffusion showed susceptible zone of inhibition. Concluding as, the ethanol extracts of Kumkum have shown appreciable antimicrobial activities against Klebsiella pneumoniae.

Key words: Phytochemical, Medicinal plants, Klebsiella pneumoniae, Kumkum, Biochemical

INTRODUCTION

Ayurveda is derived from the Sanskrit word consisting of: 'ayur'- life and 'Veda'- science/knowledge; it is characterized as both the science of life and the knowledge of life (1). Ayurveda, is the Science of Life also called the oldest healing science. It evolved in Vedic culture, and Ayurvedic remedies are mentioned in numerous Indian mythologies. These treatments are usually based on complicated herbal components, minerals, metal substances, and so forth. Metals have been used in Indian medicine since time immemorial, and they were also mentioned in Chinese and Egyptian civilizations (2).

The Devbhoomi, Uttarakhand is abundant in various plant species including several medicinal plants that are being used by locals for primary health treatments on large scale. These medicinal plants are easily available, inexpensive. Medicinal plants comprise of phytochemicals that improves the physiological balance of human beings and the knowledge of these healing properties has been passed down through generations. Phytochemical study is the interdisciplinary scientific study of biologically active agents used in traditional medicine and recently received a lot of attention (3). Phytochemical are non-nutritional bioactive substances found in plants that have therapeutic qualities. The whole world has employed medicinal plants as a key source of healthcare (4).

The mechanism of action refers to how the bioactive compounds derived from medicinal plants work against pathogenic bacteria. The mechanisms of action include: Inhibition of cell wall synthesis, Inhibition of protein synthesis, nucleic acid synthesis, enzyme activity and disruption of cell membrane integrity. The mechanism of action of these bioactive compounds is important for developing new antimicrobial agents from medicinal plants (5). Hospital-acquired infections (HAIs) are infections that occur in a patient while receiving medical care at a healthcare facility. HAIs are associated with increased

morbidity and mortality (6). Furthermore, due to the prolonged hospital stay and the need for additional treatment and diagnostic tests, HAIs increase healthcare costs. A large proportion of HAIs are caused by bacteria (7). Notably, Klebsiella pneumoniae is one of the six bacterial species that commonly cause HAIs (8). Other than pneumoniae, it also causes other miscellaneous infections such as meningitis, septicaemia, and purulent abscesses. Immunocompromised individuals have higher probability of acquiring K. pneumoniae in the lung, liver, urinary tract, blood (sepsis), and other organs. (9). It is responsible for more than 70% of infections in humans and, more worryingly, is rapidly developing resistance to multiple antibiotics. The ability to survive is attributable to biofilms which assist K. pneumoniae resist harsh host environment inside chronically infected patients (10).

The initial step in the examination of phytochemicals is extraction, which entails isolating the phytochemicals from the plant material. Solvent extraction, microwave-assisted extraction, and supercritical fluid extraction are a few of the techniques that can be used to extract phytochemicals. The plant material's raw extract is frequently put through purification processes to get rid of contaminants and isolate the desired chemicals. The extract can be purified using strategies like liquid-liquid extraction, solid-phase extraction, or chromatographic procedures (11). The different parts of the plant extract including the root, bark, and leaves have been used to treat urinary infections, nausea, syphilis, stomach ache infective wounds and other disease conditions (12). Secondary metabolites found in medicinal plants, such as alkaloids, flavonoids, saponins, tannins, and glycosides, are used to treat a variety of disease. Despite not being active in the main processes of plant metabolism, phytochemicals play critical roles in plant survival and reproduction (13). In this study we are working on Mallotus philippensis (Kumkum), a widely used medicinal herb that belongs to the family of Euphorbiaceae. It has been traditionally used as an antiviral, anticancer, anti-inflammatory, antioxidant, antibacterial, hepatoprotective, and so on and so forth. The main bioactive phytoconstituent present in the pericarp of this plant is rottlerin (14).

The identification of active compounds in medicinal plants that have antibacterial activity against Klebsiella pneumoniae can lead to the development of new drugs for treating bacterial infections. These drugs can be marketed globally and generate significant revenues for the pharmaceutical industry. The increased demand for medicinal plants can also create opportunities for sustainable harvesting and cultivation practices. This can help protect the biodiversity of the Himalayan range of Uttarakhand and ensure a steady supply of plants for the pharmaceutical and herbal medicine industries.

REVIEW LITRATURE

The northern Indian state of Uttarakhand is renowned for its abundant biodiversity and is home to a number of therapeutic plants. Traditional medicine in India has a long history and is based on various systems such as Ayurveda, Siddha, and Unani (ASU). Botanicals and medicinal plants play an important role in almost all traditional medicine. There are 2000 drugs of natural origin in Indian. According to the World Health Organisation (WHO), traditional medicines derived from medicinal plants still benefit 80% of the developing world. In comparison to the 28,187 medicinal species used by humans, the total estimated number of plants is around 374,000. WHO has also recorded the names of over 20,000 medicinal plant species and identified medicinal plants as potential sources of newdrugs. for medicinal plants Many countries have developed rules and regulations. Plant-origin products are used in approximately 60% of medicinally useful formulations and other health products. . In India, over 7800 manufacturing units are estimated to be involved in the production of natural health products and traditional plant-based formulations (15).

MATERILS AND METHODS

Bacterial strains

MTCC strain of Klebsiella pneumoniae was used, as the strains of this bacteria shows antibacterial resistance against many antibiotics.

Collection and preparation of medicinal plant sample

The plant leaves were collected based on the traditional medicinal uses and ethnomedicinal knowledge of ethnic people from different parts of Uttarakhand. The leaves of Mallots Philippensis (kumkum) were collected from Dehradun, Uttarakhand. Collected materials were washed with running tap water and later with sterile water to remove all the dirt. Leaves were shade dried for 12-15 days by protecting them from direct sunlight to prevent the degradation of active ingredients followed by grinding into fine powder.

Preparation of plant extract by maceration process

10 gms of dried powder sample was mixed with 100ml of 70% ethanol and 70% methanol in conical flask as solvents for cold maceration procedure. The extracts were kept in shaking conditions at 37 ℃ for 48-72h. The mixture was passed through a mesh sieve (1 mm), filtered using Whatman filter paper no. 1, and then evaporated at 40-50℃ using a rotary evaporator. The final extract was measured and the working solution was prepared in 50% DMSO. The plant extracts stock solutions were maintained at 4 °C in the refrigerator until use. The final concentration of the extract was 1gm/100ml (fig 1) (16)

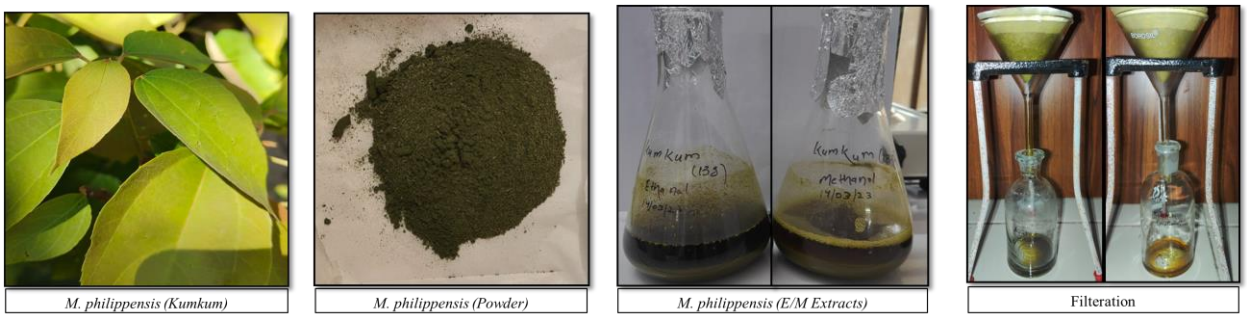

Figure 1: Preparation of methanol and ethanol extract M. philippensis leaves

Phytochemical analysis of Mallots philippensis leaves extract

Phytochemical tests are laboratory procedures used to identify and analyze the presence of bioactive compounds in plants, also known as phytochemical. These tests help determine the chemical composition of plants and can provide valuable information about their potential health benefits and therapeutic properties. These tests provide preliminary information about the presence of specific phytochemical but do not determine their exact identity or concentration. The obtained extracts were subjected to phytochemical screening using standard methods
(17)

Test for Alkaloids

Mayer's Test 2ml extract (1ml extract diluted with 1ml distilled water) was added into test tube then few drops (7-10) of Mayer's reagent was added into the test tube. Yellowish/ White precipitates will be formed if alkaloids are present.

Test for Saponins

1ml extract was taken in test tube with 5ml of distilled water. The extract was then vortexed for few minutes. Presence of foam produced persists for 10 min, it confirms the presence of saponins.

Test for Flavonoids

1ml extract was taken in test tube. Then 2 ml of 2% NaOH was added into the test tube. After the appearance of intense yellow colour, add few drops of 10% dil. HCL. If the solution turns colourless, it indicates the presence of flavonoids.

Test for Tannins

2ml extract (1ml extract diluted with 1ml distilled water) was added into test tube then 2 ml of distilled water was added. 5-6 drops of 5% FeCl3 were added into the extract. Blue/Black/Green precipitate indicates the presence of tannins.

Test for Proteins

Ninhydrin Test 1 ml of extract was mixed with 2ml of 0.2% solution of Ninhydrin and boiled. A violet colour precipitate appeared suggesting the presence of amino acids and proteins.

Milon's Test

To 2 ml of filtrate few drops of Millon‟s reagent was added to 2ml of plant extract. A white precipitate indicates the presence of proteins.

Test for Resin

2ml extract (1ml extract diluted with 1ml distilled water) was added into test tube then 2ml of distilled water was added. 1ml acetone was added to the extract. Addition of acetone resulting in turbidity of the extract indicates the presence of resin.

Test for Phenols

2ml extract (1ml extract diluted with 1ml distilled water) was added into test tube then 3ml of 10% NaOH solution was added into the extract. Appearance of yellow colour indicates the presence of phenols in the extract.

Test for Carbohydrates

Fehling's Test 2ml extract (1ml extract diluted with 1ml distilled water) was added into test tube. Then 5ml of Fehling's solution (2.5ml of Fehling's solution A and 2.5ml of Fehling's solution B) was added into the extract. The solution was boiled over heating plate for 5mins. Formation of red precipitate indicates the presence of carbohydrates in the extract.

Benedict's Test 2ml extract (1ml extract diluted with 1ml distilled water) was added into test tube. 2ml of Benedict's reagent was added into the extract. Then the mixture was heated on a boiling water bath for 5 min and cooled. Formation of orange red precipitates indicates the presence of carbohydrates.

Antimicrobial activity of Mallots philippensis extract

The disk diffusion method was used to investigate the antimicrobial activity of Mallots philippensis (kumkum) leaves extracts against Klebsiella pneumoniae strain (MTCC 109). The bacterial strains cultures were acquired from the Microbial Type Culture Collection and Gene Bank, Chandigarh and subcultured onto the flesh Macconkey agar. Few colonies were picked from the culture to prepare the bacteria suspension of each strain to be tested and inoculated in broth for primary culture. An overnight well grown culture was used for spreading the Muller Hinton Agar media (MHA) plate to be tested. Paper

discs (5 mm) prepared from Whatman filter papers were placed on top of the innoculated plates. The M. philippensis leaves with ethanol and methanol extract were tested for their inhibitory activity against the bacteria by pippeting different volume (40, 45, 50 and 55 µl) of 1% solution of each onto the 5 mm paper discs. Distilled water and solvent were used as control for comparison. All plates were incubated overnight at 37˚C. The zones of inhibition were observed, and measured including the 5 mm sized discs, and recorded (18).

Antibiotics sensitivity assay against K. pneumoniae

100 µl of overnight bacterial culture was taken using micropipette and transferred into NAM plates for spreading. The plates were dried and then used for the antibiotic sensitivity test. Antibiotic disks were purchased from CLAIRO COMBI. Using sterile forceps, antibiotic disc (negative and positive) was placed on the surface of the same plate. Immediately discs were pressed down lightly with the forceps. Plates were incubated overnight at 37°C. The zones of inhibition were observed and measured using scale.

Statistical Analysis

All the experiments were performed in triplicate (n =3). The significant results were shown in result section.

RESULTS AND DISCUSSION

Screening of phytochemical from different extracts of Mallots philippensis leaves

The phytochemical screening was achieved through biochemical testing on different extracts (methanol, and ethanol) of Mallots philippensis leaves to detect the presence of medicinally active components. This screening test showed different results in different extracts of Mallots philippensis leaves as shown in fig 2 and Table no. 1. Significant indication about the presence of tannins, saponins, proteins, alkaloids, carbohydrates, phenol and resin was shown in both the extracts, whereas flavanoids was not present in any extract.

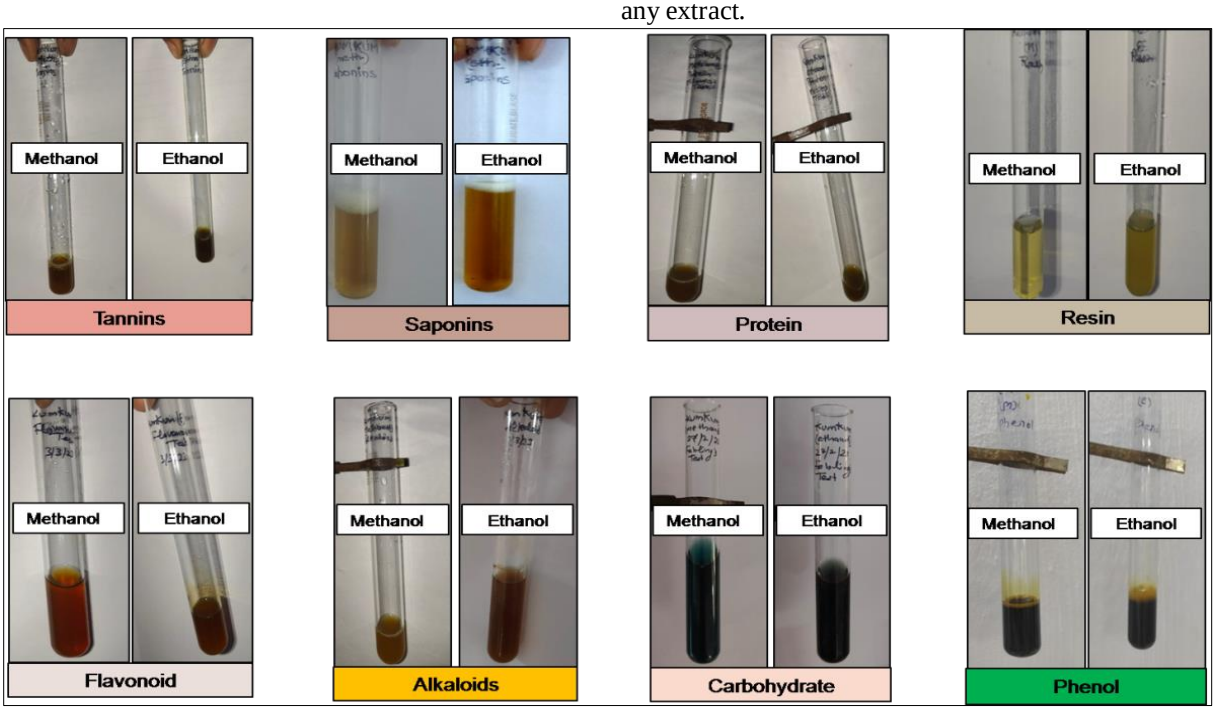

Figure 2: Phytochemical analysis of M. philippensis (kumkum) methanol and ethanol

Table 1: Results of phytochemical screening of methanol, and ethaol extracts of Mallots philippensis leaves

S. no.	Phytochemicals/ Plants	Tannins	Flavanoids	Saponins	Proteins	Alkaloids	Carbohydrates	Phenol	Resin
1	*Mallots philippensis* (Kumkum) (M)	++	-	++	++	+	++	+	++
2	*Mallots philippensis* (Kumkum) (E)	++	-	+	++	+	++	+	++

The results of the present study showed that M. philippensis leaves are great reservoirs of medicinally active ingredients (phytochemicals) and they are consistent with earlier phytochemical screening studies done on different solvent extracts which also reported that M. philippensis leaves extracts are rich in the above-mentioned phytoconstituents. Bioactive constituents such as alkaloids, phenols, saponins and tannins etc are known to elicit broad antimicrobial responses against bacteria, fungi, viruses and parasites (19). A number of studies documented that these phytochemicals also possess many pharmacological properties, including anticancer, anti-inflammatory, cytotoxicity, antioxidant, anti-hemostatic, anti-diarrheal, anti-hemorrhoidal, anti-apoptosis and growth regulation (20).

Antimicrobial activity of M. philippensis leaves extract against K. pneumoniae

Ethanol and methanol extract of M. philippensis leaves were tested for their antimicrobial activity against the bacteria using various volume (40, 45, 50 and 55 µl) of 1% solution of each extracts. Distilled water and solvent were used as control for comparison. Our result indicates that 40 µl of ethanol extract showed resistant effect with 10 mm zone of inhibition (ZOI), 45 µl of ethanol extract showed intermediate with 12 mm ZOI, whereas, 50 and 55 µl of ethanol extract was sensitive against K. pneumoniae. Interestingly, no inhibition zone was observed in case of methanol (Fig 3 and Table 2). Furthermore, we observed that ethanol extracts showed higher antibacterial activity significantly.

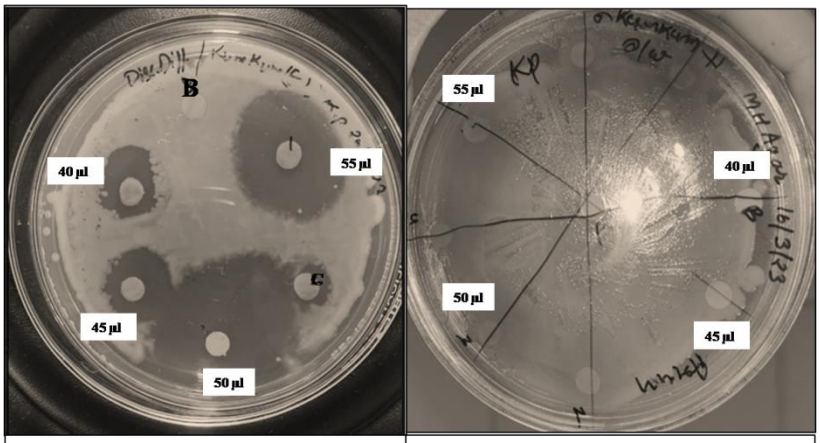

Figure 3: Inhibitory effects of different preparations/extracts of M. philippensis leaves extract against K. pneumoniae strain

Table 2: Zones of inhibition of different preparations of M. philippensis leaves extract treatment against K. pneumoniae

S. No.	Plant Extract	Volume in µl	Zone of Inhibition	Interpretation
1	M. philippensis (E)	40	10 mm	Resistant (R)
		45	12 mm	Intermediate (I)
		50	22 mm	Sensitive (S)
		55	25 mm	Sensitive (S)
2	M. philippensis (M)	40	NZI	Resistant (R)
		45		
		50		
		55		

Antibacterial activity of known antibiotics against K. pneumoniae

The antibacterial activity of antibiotic (for gram negative and positive bacteria) was assayed by disc diffusion method against K. pneumoniae (fig 4). The microbial growth inhibition of antibiotics summarizes in table no.3

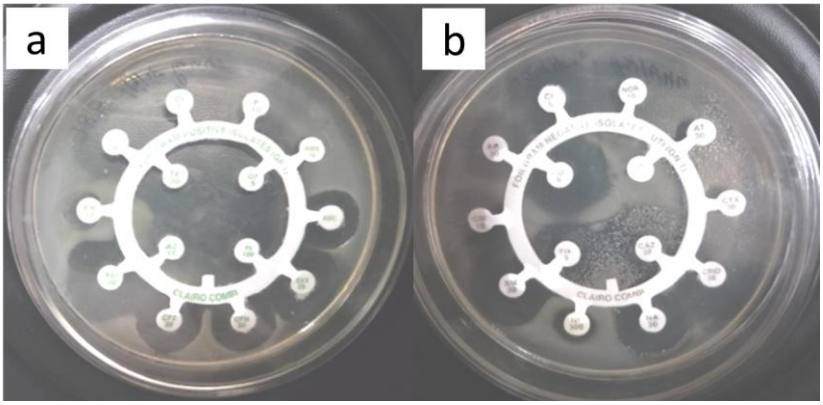

Figure 4: Antibacterial activity of antibiotics (a) Gram Positive and (b) Gram negative against Klebsiella pneumoniae

Table 3: Antibacterial susceptibility of antibiotics (Gram Positive and Gram negative) against K. pneumoniae

S. No.	Gram positive Antibiotics	Zone of Inhibition (mm)	S. no.	Gram negative Antibiotics	Zone of Inhibition (mm)
1	Penicillin-G (P/10)	-	1	Norfloxacin (NOR/10)	30
2	Amoxicillin (AMX/10)	-	2	Aztreonam (AT/30)	-
3	Amoxicillin-C (AMC)	17	3	Cefotaxime (CTX/30)	-
4	Co-trimocazole (SXT/25)	22	4	Ceftriaxone (CRO/30)	18
5	Cephalexin (CFM/30)	17	5	Nalidixic acid (NA/30)	18
6	Cefazolin (CFZ/30)	17	6	Nitrofurantoin (NI/300)	11
7	Cefuroxime (XM/30)	25	7	Cefuroxime (XM/30)	21
8	Erythromycin (EM/15)	-	8	Gentamycin (GM/10)	14
9	Chloramphenicol (C/30)	21	9	Amikacin (AK/30)	16
10	Ciprofloxacin (CI/5)	26	10	Ciprofloxacin (CI/5)	20
11	Ofloxacin (OF/5)	24	11	Ofloxacin (OF/5)	-
12	Piperacillin (PI/100)	30	12	Ceftazidime (CAZ/300	-
13	Azinthromycin (AZ/15)	13	13	Cefixime (FIX/5)	-
14	Tetracycline (TE/30)	23	14	Cefdinir (CD/5)	23

As shown in the result of Gram positive antibiotic penicillin-G, amoxicillin, and erythromycin were sensitive against K. pneumoniae, whereas other antibiotics like Co-trimocazole, Cephalexin, Cefazolin, Cefuroxime, Chloramphenicol, Ciprofloxacin, Ofloxacin, Piperacillin, Azinthromycin, and Tetracycline were resistant. Furthermore, the effect of gram-negative antibiotics like Norfloxacin, Ceftriaxone, Nalidixic acid, Cefuroxime, Amikacin, Ciprofloxacin, and Cefdinir showed significant resistant (>16mm) against K. pneumoniae. On the other hand, no inhibition zone was observed in the antibiotics Aztreonam, Cefotaxime, Ofloxacin,, Ceftazidime, and Cefixime.

CONCLUSIONS

The characterization of the phytochemical composition and antimicrobial activity of M. philippensis (kumkum) leaves was carried out using methanol and ethanol extracts. Medicinally active components of interest from M. philippensis leaves were successfully extracted using these solvents, and identified. The phytochemical screening investigations found most of the major bioactive phytochemicals in different extracts and this ascertained their medicinal value. Many effective antibiotics showed resistant results against K. pneumoniae. In general ethanol extracts inhibited bacterial growth more effectively as compared to methanol extracts. Interestingly no inhibition zone was observed in the case of methanol extract. Hence, the ethanol extract of M. philippensis leaves were significantly effective against K. pneumoniae MTCC strain.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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