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ORIGINAL RESEARCH ARTICLE

Phytochemical Investigation and Anti Microbial and Enzyme Inhibition Activity of *Murraya Koenigii* (Linn).Spreng

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ABSTRACT

To investigate the antibacterial and antifungal activity of insolated compounds by column chromatography obtained from *Murraya Koenigii* stem bark extracts. The isolated compounds of *Murraya Koenigii* (Su-I, Su-II, Su-III and Su-IV) were tested for antibacterial efficacy against *staphylococuus aureus* and antifungal efficacy against *candida albicans*. The antimicrobial effect produced by the isolated compounds was comparable to that of Amikacin and Griseofulvin. The isolated compounds (Su-II, Su-III and Su-IV) were found out in amylase and urease enzyme. The isolated compounds (Su-III and Su-IV) showed high enzyme inhibitory activity.

Keywords: *Murraya Koenigii,* Antimicrobial activity, Enzyme inhibitory activity, Phyto chemical investigation.

INTRODUCTION

Murraya Koenigii (Linn), Rutaceae family is found in common in forests often as gregarious undergrowths. The plant used as tonic, stomachic and carminative. Fresh juice of the root is taken to relieve pain associated with kidney. Leaves are used internally in dysentery and diarrhea. The aqueous extracts of leaves, when administered parentrally to female guinea pigs, not only raised the phagocytic index but also mobilized a greater number of leucocytes to take part in phagocytosis. The green leaves are described to be eaten raw for the cure of dysentery [1, 2, 3, 4, 5]. In Ceylon a decoction of the leaf is given internally in snake bite. In Indochina the fruit is considered astringent. *Murraya koenigi* is an aromatic more or less deciduous shrub or a small tree up to 6 m in height and 15-40 cm in diameter found throughout India up to an altitude of 1,500 m commonly in forests often as gregarious under growths. It is cultivated for its aromatic leaves. The plant has been identified by Dr. Stephen, Dept. of Botany, American College Madurai. Root and bark are stimulant and are applied externally for skin eruptions and poisonous bites. Green leaves are febrifuge and are used in dysentery. The whole plant is considered to be a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys. In the light of the above information the present investigation was under taken which deals with the studies of the isolated compounds by Column chromatography against Gram+ bacteria and Fungi *Candida albicans*, the result of which are being reported win the present communication. The enzyme inhibitory activity was found out by using amylase and Urease enzyme.

MATERIALS AND METHODS

Plant materials

Whole dried stem bark of *Murraya Koenigii* (Linn) were collected in the of July, Madurai district of Tamilnadu, India. Taxonomic identification made from Dr.Stephen, Botanist, American College, Madurai. The stem bark dried in the shade. Then the shade dried stem barks were powered to get a coarse powder.

Preparation of extracts

Dried coarse powder of the stem bark (1kg) was placed into the extractor of a Soxhlet apparatus and subjected to extraction by hot percolation

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method. The extraction was carried out by using solvents of increasing polarity starting from petroleum ether, chloroform and acetone. The extraction was carried out with 2 liters of each solvent for a period of 72 hours. At the end of the extraction the respective solvents were concentrated by evaporation.

Preliminary phytochemical investigation

The qualitative chemical test of various extracts of *Murraya Koenigii* was carried out using standard procedure [6, 7, 8, 9]. Carbohydrate, sterols, Coumarins, Flavonoids and Alkaloids are present in the extracts.

Table1: Preliminary phytochemical investi Gation of *Murraya Koenigii*

Name of extract	Sugar	Flavanoid	Coumarin	Alkaloid	Sterol	Glycosides
Petroleum ether	+	-	-	+	+	-
Chloroform	-	-	+	+	+	-
Acetone	-	-	+	+	-	-
Alcohol	+	+	+	-	-	-

Preparation of column chromatography

After screening the various extracts obtained from 1 kg of coarse bark powder, the petroleum ether extract and chloroform extract were found to be promising. The petroleum ether (8gm) was a dark green residue. He chloroform extract was dark brown residue (20gm) were pooled and chromatographed over silica gel (100-200mesh). Then the compounds separated starting with solvents like n-hexane, pet.ether, benzene, chloroform, acetone, ethyl acetate and methanol and the compounds isolated as (Su -I,Su-II, Su-III and Su-IV)

Enzyme inhibitor activity [10, 11, 12]

Preparation of the enzyme

1. Salivary amylase

10ml of the saliva was collected and diluted to 100ml with cold phosphate buffer pH 7.1. The solution was centrifuged at 8000 rpm for 20mts and the clear supernatant was used.

2. Extraction of crude inhibitor

There are several reports on the extraction of enzyme inhibitors from plant materials. We have adopted the method Buonocore et.al 100mgs of dried plant powder was extracted with 2.5% of cold TCA with magnetic stirrer for 45mts. The solution was centrifuged to get a clear supernatant, which was neutralized to pH 7 with dilute sodium carbonate and used for the assay of enzyme inhibition. This is referred as the crude inhibitor.

Amylase assay

Amylase activity was determined by the method of Bernfeld 1ml of the enzyme solution was added to 2ml of phosphate buffer pH6.9 containing 2MNacl and the reaction started with the addition of 2ml 1% soluble starch solution. The tubes were incubated at 37°C fir 20mts. The reaction was arrested by the addition of 1ml for dinitrosalicylic acid colour reagent. The tubes were kept in a boiling water bath for 10mts, cooled and diluted t a final volume of 10ml with distilled water. The absorbance was measured at 530nm in an Erma photoelectric colorimeter. Amylolytic activity was calculated as maltose equivalents liberated. One unit of enzyme is defined as the milligrams of maltose liberated per minutes under the assay conditions. The standard curve was constructed using pure anhydrous maltose. The assays were run along suitable blank (without enzyme).

To measure the amylase inhibitory activity, suitable amount of 0.1% mercuric chloride was pre incubated with the enzyme, and the assay was carried out as described above. The inhibition caused by 0.5% of mercuric chloride was arbitrarily fixed as 100% inhibition. Suitable amounts of plant extract were similarly tested for the presence of amylase inhibitors in the extract. The decrease in the inhibitory activity of the crude plant extract was defined on the basis of these parameters. The crude extracts showed about 80-100% inhibition

$$\% \text{ inhibition} = \frac{\text{Abs of control (no inhibitor)} - \text{Abs of sample}}{\text{Abs of control (no inhibitor)}} \times 100$$

The crude extracts inhibitory activity was not destroyed when heated to 80°C, thus revealing its likely non proteinaceous nature. From these observations it can be inferred that further purification can lead to more information on these natural enzyme inhibitors.

3. Assay of Urease

Urease enzyme was prepared from horse gram seeds. The assay of enzyme was carried out according to the method described by Malhothra using Nessler's reagent. A standard was prepared using NH_4SO_4.urease inhibition was compared with parachloromercuric benzoate (PCMB), the activity of which was set arbitrarily to 100%. Under these conditions the crude extract showed about 100% of inhibition.

Phytochemical investigation

Spectral data for isolated compounds

Compound A (Su – I)

Su – I, crystallized from n-hexane as yellow amorphous solid, showing a melting point at 170°C and R_f value 0.8181. (Solvent system petroleum ether: chloroform) 9: 1. The UV absorption spectra of Su – I increases from λmax 277, 288, 300, 310 nm is characteristics of carbozole skeleton. IR spectra of Su – I revealed the presence of prominent peak at 2923 cm^{-1} may be due to C-H stretching vibration. The peak at 3399 cm^{-1} may be due to N-H stretching vibration. A strong band at 1126 cm^{-1} corresponds to C-O stretching vibration. The NMR spectra of the compound showed singlet at δ2.2 corresponding to methyl protons linked to aromatic ring. Double at δ3.5 and singlet at δ3.9 corresponds to methylene protons adjacent to oxygen. A singlet at δ7.7 and doublets at δ6.8 – 7.3 corresponds to aromatic protons.

Compound B (Su – II)

Su – II, crystallized from chloroform as greenish powder, melted at 140° C and R_f value 0.84615. (Solvent system petroleum ether: chloroform) 9.8: 0.2. The UV absorption spectra of Su – II increases from λmax 277, 288, 300, 310 nm is characteristics of carbozole skeleton. IR spectra of Su – II revealed the presence of a prominent peak at 2925, 2846 cm^{-1} due to C-H stretching vibration. The peak at 3397 cm^{-1} may be due to N-H stretching vibration. The NMR spectra of the compound showed singlet at δ2.2 and at δ7.3 corresponds to methyl and aromatic protons respectively.

Compound C (Su – III)

Su – III crystallized from acetone as dark brown residue, melted at 120° C and R_f value at 0.6481 solvent system Chloroform : methanol 9.8 : 0.2.The UV absorption spectra shows a broad maximum at 260, 300, 310 and 320 nm which is a characteristics of 5,7-di-methoxy coumarins. The IR spectra revealed the presence of a prominent peak at 2929 cm^{-1} and 2857 cm^{-1} due to C-H stretching vibration. The presence of a strong band at 1668 cm^{-1} and 1619 cm^{-1} (α,β-unsaturated ketone) due to C=O stretching vibration. A prominent peak at 1276 and 1137 cm^{-1} corresponds to C-O stretching vibration.The NMR spectrum of the compound shows at δ1.6 corresponds to side chain methylene protons. A singlet at δ7.3 corresponding to aromatic protons and singlet at δ2.2 corresponding to methyl proton adjacent to carbonyl group.

Table 2: Enzyme inhibitory activity of *Murraya Koenigii*

Enzyme	Standard inhibitor	Extracts (percentage on inhibition)				
		1	2	3	4	5
Amylase	Mercuric chloride	100%	Nil	Nil	trace	100%
Urease	Para chloro mercuric benzoate	100%	Nil	Nil	trace	100%

1. Standard inhibitor
2. Su-I
3. Su-II
4. Su-III
5. Su-IV

Evaluation of Anti microbial activity

Antibacterial activity [13, 14, 15, 16]

Assay was carried out by diffusion plate method. The method followed was spread plate technique. The plates free from contamination were spread with 50µl of 48h old culture of bacterial test organism using sterile buds. The standard disc of Amikacin (sterile) of 5 mm diameter was in the Petri plates. Then the filter paper discs (sterile) of 5mm were soaked in 1ml (1µg/ml) of the test solution and in solvent control DMF. After evaporating the solvent in a sterile atmosphere the drug impregnated discs were placed in Petri plates. The plates were refrigerated for 1h to arrest the growth and for easier diffusion of test compounds. Then the plates were removing from refrigerator and incubated at 37^0C over night is an inverted position. The clear zones of inhibition were measured using Hi media zone reader scale. The values are tabulated. The zones of test solutions were compared with standard Amikacin.

Table 3: Evaluation of Antibacterial activity of *Murraya Koenigii*

SlNo.	Compounds	Microorganism used (Staphylococcus aureus) Zone of inhibition in (mm)
1	Standard (amikacin)	11
2	Su-I	10
3	Su-II	20
4	Su-III	22
5	Su-IV	18
6	Chloroform extract	9
7	Carbazole alkaloid(std)	8

Table 4: Evaluation of Antifungal activity of *Murraya Koenigii*

SlNo.	Compounds	Microorganism used (Candida albicans) Zone of inhibition in (mm)
1	Standard (griseofulvin)	11
2	Su-I	12
3	Su-II	19
4	Su-III	21
5	Su-IV	18
6	Chloroform extract	11
7	Acetone extract	20

Antifungal activity

Glucose, peptone and agar were taken in the above proportions and dissolved upto 1000 ml of distilled water. The constituents were heated gently at 100° C with agitation. The pH of the medium was adjusted to 5.4. Then it was transferred to boiling tubes in hot condition and sealed with non-absorbent cotton and sterilized by autoclaving at 121° C (15 lbs pressure) for 15 mts. Then poured aseptically into sterile Petri dishes. The temperature of the medium should not exceed above 50° C when the organisms were inoculated. The standard drug Griseofulvin (10µg/disc) was placed on the media. The sterile whatmann no.2 filter disc (5mm diameter) was soaked in synthesized compounds (200µg/disc) separately and evaporated to dryness and then kept on the media. One more disc immersed in dimethyl formamide and kept on the media as control. The Petri dishes were incubated at 37° C for 24hrs, after placing them in the refrigerator for one hr to facilitate uniform diffusion. Observations were made for the zone of inhibition around the synthesized compounds with that of standard.

RESULTS AND DISCUSSION

Su-I has no considerable antibacterial activity when compared to standard against *S.aureus*. it showed moderate antifungal activity. Su – II showed very high antibacterial and antifungal activity when compared with the standard drug. Su – III which was identified as a coumarin also showed high degree of antibacterial and antifungal activity. This compound exhibited high degree of amylase and urease activity. Su – IV showed very high degree of both antibacterial and antifungal activity. The significant feature of this extract is very high amylase inhibitory activity even in low concentration. Disc diffusion methods are used extensively to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of the substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. Due to limited capacity of discs, holes or cylinders are preferably used. Most of the bacterial species and the fungal species were inhibited by the plant extract. In this study, one bacterial and fungal species were used to screen the possible antimicrobial activity of the extract. It showed a broad spectrum of activity against all the bacterial strains. Amikacin and Griseofulvin were used as positive controls for bacteria and fungi, respectively. As reported earlier secondary metabolites like flavonoids, alkaloids are likely responsible for the observed antibacterial activity of plants [17, 18, 19]. The presence of said constituents in extract of *Murraya Koenigii* as found in phytochemical test may be responsible for the antibacterial activities.

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