Muntingia Calabura: Potential Source of Pharmacologically Active Substances

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Abstract- Plants are generally measured as origin of natural drugs and are extensively used in herbal formulations. Muntingia calabura (Muntingeaceae) is indigenous to Central America and Southern Mexico It is abundantly disseminated throughout Asian countries especially in India. The present research was done on the isolation, pharmacological study of the isolated compounds derived from root heart wood and bark of the root of Muntingia calabura. For this study, six compounds were taken into account and later it was divided into two categories as flavonoids and chalcones. The isolated compounds were further screened for their attributed pharmacological traits. All the compounds screened noticed to possess significant medicinal properties.

Key Words: Anti-oxidant, anti-inflammatory, anti-microbial, flavonoids, chalcones.

I. INTRODUCTION

Since, historical stand point, plants have been major source of crude drugs and herbal formulations that represent 25% of all the drugs for medicinal purpose [1]. Natural drugs with potential therapeutic properties have been the basis in drug discovery and sdevelopment [2]. Advanced developments in the areas of X-ray Crystallography [3], NMR spectral analysis [4] and alternate method developments for drug discovery viz., combinatorial chemistry and rational drug design are the key factors for the drastic increase of drug discovery from higher plants in recent years. However, despite of these well developed alternative methods, yet there is a flaw of lead compounds advanced towards clinical examination.

India is bestowed with variety of plant attribute for medicinal properties. By the literature

view, it has been clear that approximately 3000 plants of India have sure ccessfully screened and reported their therapeutic applications. The number of these plants may increase 50% by 2020.

Among various medicinal plants *Muntigia calabura* is one of such therapeutically valuable plant and reported for several pharmacological properties [5]. However, medicinal properties of this plant require proper scientific evidence documentation before its use. Hence, the current studies have been focused for the isolation of pharmacologically active substances form heart wood of root and root bark

II. MATERIALS AND METHODS

A. Plant Material

The heartwood of root and root-bark were used as the materials, cut into smaller pieces, and pulverized into rough pow der.

B. Chemicals

Gentamycin sulphate, Deoxyribose, 2, 2'-azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS*). Nitrobluetetrazolium (NBT), Riboflavin, L-methionine, thiobarbituric acid, Ethylenediamine tetra acetic acid (EDTA), ascorbic acid, ferric chloride anhydrous ($FeCl_3$) potassium persulfate, trichloro acetic acid, Vitamin C.

C. Bacterial Strains

Strains of *Staphylococcus aureus* ATCC 96, *Bacillus subtilis* MTCC 441, *Bacillus cereus*, ATCC 9372, 09, *Klebsiella pneumonia* MTCC 109, *Escherichia coli* ATCC 8739, *Proteus vulgaris* KUCC 21. Particular media nutrient broth was used for the growth of bacteria at 37^{o}C and maintained on nutrient agar slants at 4^{o}C.

D. Preparation of standard bacterial suspensions

The vaccinated strains of bacteria were nourished at 35 ± 2°C for 24 h. The turbidity results in suspensions were diluted with same nutritive broth transmittance of 25% at 580nm was obtained. Further, Bausch & Lomb spectrophotometer was used to calculate the percentage spectrophotometrically. The stock solutions of ampicillin, azithromycin, were prepared at 10 mg/mL in DMSO.

E. Separation of biomolecules

Extraction of compounds

The shade dried heart-wood and bark of the root of *Muntingia calabura* was subtly powdered (500g), The chloroform was used as extracted solvent in soxhlet apparatus. Residue remained after evaporation was further processed for bioassay guided fractionation.

Bioassay guided Fractionation of extracts

After evaporation crude extracts of *Muntingia calabura* were subjected to a silica gel column (100-200 mesh). Initially benzene and chloroform (100:0) was used as a solvent in a stepwise gradient for elution. and ending with benzene: chloroform (0:100).

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The fractions were collected, concentrated under reduced pressure. Each fraction was screened for anti-bacterial, activity.

Fractions activities were identified by using percolated TLC plates. Benzene and ethyl acetate (5:3) used as solvent system. Iodine vapors were applied in order to visualize the spots. Fractions that exhibited immense activity were subjected to a second time. These fractions are further purified to achieve greater separation. Depiction of the separated compounds was done carefully and without interfering of some non-target compounds.

G. Determination of anti-bacterial activities of isolated and characterized compounds

According to Raman (2009), method was used for the antibacterial activity of the purified compounds [6]. Gentamycin sulphate (10µg/ml) was used as positive control. The test was conducted for three times

H. Determination of anti-fungal activities of isolated and characterized compounds

Preparation of sample

In DMSO test samples were prepared with various concentrations as 50 µg/ml, 100 µg/ml, and 150 µg/ml.

Spore suspension Preparation

spores were gathered and uprooted from the fresh cultures, in a test tube consists in sterilized Sabourad's dextrose broth. In order to test the antifungal activity the spore suspension was used.

Anti-fungal assay

Magaldi (2004) methods discussed to test the anti-fungal activity of the test compounds agar well diffusion method was used [7]. The open wells were filled with 25 µl of each compound at various concentrations (50, 100, 150 µg/ml). Test was carried out on Asthana and Hawker's Medium plates and incubated at 22^0C for 72 hrs. The zones of inhibition were estimated and compared with reference drug nystatin.

I. Assay of anti-oxidant property

Lipid peroxidation assay

Satohet *et al*., 1978 and Bouchet *et al*., 1998, method was used to determine *the In vitro* lipid peroxidation of the compounds[8,9].

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging activity

1,1-diphenyl-2-picryl- hydrazil (DPPH) method was used for the free radical scavenging activity of isolated compounds [10].

$$\%\ DPPH\quad radical\quad scavenging\quad = \frac{(Absorbance\ \ of\ control - Absorbance\ \ of\ \ test\ sample)}{Absorbance\ \ of\ \ control} \times 100$$

J. ABTS*+ (2, 2'-azino-bis (3-ethylben - zthiazoline-6-sulphonic acid) radical cation decolourisation assay

This method was carried out by well described method Pellegrini [11].

K. Anti-inflammatory activity

The anti-inflammatory activities of active fractions of *Muntingia calabura* were examined by the described method [12]. The % of inhibition of protein denaturation was calculated using the following formula:

$$\%\ inhibition = 100\left(\frac{V_t}{V_c} - 1\right)$$

Where, Vt = absorbance of test sample,

Vc = absorbance of control.

By plotting percentage inhibition with respect to control versus treatment concentration the IC_{50} values were calculated.

II. RESULTS

A. Fractionation of extracts and bioassay guided fractionation

Based on bioassay guided fractionation, ^{1}H NMR and ^{13}C spectral data, one compound was identified as chalcone from five which are isolated from the current plant (Table 1). 8-hydroxy-7,3′,4′,5′-tetramethoxy flavone **(1),** 8,4′-Dihydroxyl-7,3′,5′-trimethoxyflavone **(2),** 8-methoxy-3′,5′7′-trihydroxyflavone **(3)**, 3,5,7-trihydroxyflavone (Galangin) **(4),** 5,8-dihydroxy-6,7,4′trimethoxy flavones **(5)**, 6,4′ dihydroxy 3′ propen chalcone **(6)** and possess the molecular weight $C_{19}O_7H_{18}$, $C_{18}O_7H_{16}$, $C_{21}H_{20}O_4$, $C_{21}H_{25}O_4$, $C_{18}H_{16}O_7$ and $C_{18}H_{16}O_3$. The isolate compounds structures were shown in figure 1.

B. Determination of anti-bacterial activities of isolated and characterized compounds

Compounds isolated exhibited good anti-bacterial activities (Table 2). Amid the tested samples, 8- methoxy-3′, 5′7′-trihydroxyflavone showed highest inhibitory zone 18 mm against methicilin resistant Staphylococcus aureus (MRSA) at 150 µg/ml (Fig 2). Whereas, 3,5,7-trihydroxyflavone (Galangin) was the next effective compound against to MRSA with inhibition zone of 15 mm recorded at 150 µg/ml (Fig 2). However, all the tested organisms are more susceptible these compounds. Whereas, compounds 8- hydroxyl-7,3′,4′,5′-tetramethoxyflavone, 8,4′-Dihydroxyl-7,3′,5′-trimethoxyflavone, 5,8-dihydroxy-6,7,4′trimethoxy flavones and 6,4′ dihydroxy 3′ propen chalcone are also exhibited considerable activity (Table 2). Reference drug gentamycin sulphate at 10µg/ml was noticed immense anti-bacterial potential than gram negative strains comparing with that from gram positive strains.

C. Determination of anti-fungal activities of isolated and characterized compounds

3,5,7-trihydroxyflavone (Galangin) was noticed highest anti-fungal activity compared with that from 8- methoxy-3′, 5′7′-trihydroxyflavone by producing 10 mm inhibitory zone against *aspergillus niger* at 150 µg/ml (Fig 2). Whereas, 8-methoxy-3′, 5′7′-trihydroxyflavone was more effective compare to *Candida albacans* with inhibition zone of 9 mm at 150 µg/ml. Although, 8- hydroxyl-7,3′,4′,5′-tetra methoxy flavone was failed to possess anti-fungal activity against *Candida albacans*. Table 3 represents anti-fungal activity of the tested compounds compared with reference drug Nystatin at 10µg/ml.

D. Assay of antioxidant property

Lipid peroxidation assay

8-hydroxyl-7,3′,4′,5′-tetramethoxyflavone and 8,4′-Dihydroxyl-7,3′,5′-trimethoxyflavone exhibited concentration-dependent $FeSO_4$ induced lipid peroxidation and showed highest percentage of inhibition with that from other compounds and comparable to reference drug BHA. P< 0.5 (Table 4).

DPPH radical scavenging activity

Among the tested compounds 8-hydroxyl-7,3′,4′,5′-tetramethoxyflavone and 8,4′-Dihydroxyl-7,3′,5′-trimethoxyflavone are showed highest percentage of inhibition which was comparable with standard BHA. 8- methoxy-3′, 5′7′-trihydroxyflavone, Galangin, 5,8-dihydroxy-6,7,4′trimethoxy flavones and 6,4′ dihydroxy 3′ propen chalcone are also possessed considerable anti-oxidant properties P<0.5 (Table 4).

$ABTS^{*+}$ radical cation decolourisation assay

Isolated compounds exhibited good scavenging of $ABTS^{*+}$ radical at all tested concentrations (Table 4). The highest inhibition was achieved with 3,5,7-trihydroxyflavone (Galangin) comparing to other compounds P<0.5. Whereas, 8- methoxy-3′, 5′7′-trihydroxyflavone was the next compound showed significant at 150 µg/ml (Table 4).

E. Anti-inflammatory activity

Among various compounds tested 8,4′-Dihydroxyl-7,3′,5′-tri methoxy flavone exhibited effective inhibition of protein denaturation and at 150µg/ml 95 % which is very much near and more comparable with reference drug Diclofenac sodium 99 % at 20µg/ml concentration (Fig 3). On the other hand, 8-hydroxyl-7,3′,4′,5′-tetra methoxy flavone also exhibited good inhibition of protein denaturation. The inhibition percentages noticed by these compounds at 50, 100, 150 µg/ml concentrations are 51, 71, 95 and 30, 56, 78 respectively. The IC_{50} values 51 and 56 are obtained for both the compounds. Percentage of protein denaturation inhibition at 50, 100, 150 µg/ml of the compounds are depicted in Fig 2.

IV. DISCUSSION

Most of the natural products in drug exploration have been derived from the distinct medicinal plants in the terms of secondary metabolites. These are frequently recognized [13]. Plant ingredients play a key role as of prescribed drugs or herbal preparations and are utilized for the treatment of 87% of human diseases [14].

With the connectivity to plant obtain drugs, the ongoing research has been accomplished for the isolation of pharmacologically active compounds from *Muntingia calabura* (Muntingiaceae).Use of *Muntingia calabura* crude extracts requires a proper scientific evaluation and documentary for the isolation of active principle. The compounds isolated from this plant are identified as flavonoids and chalcones. Among, separated compounds 8-hydroxy-7,3′,4′,5′-tetramethoxy flavone **(1),** 8,4′-Dihydroxyl-7,3′,5′-trimethoxyflavone **(2),** 8-methoxy-3′,5′7′-trihydroxyflavone **(3)**, 3,5,7-trihydroxyflavone (Galangin) **(4),** are previously reported from the same plant [15]. Whereas, 5,8-dihydroxy-6,7,4′trimethoxy flavone **(5)** and 6,4′ dihydroxy 3′ propen chalcone **(6)** are also known compounds but were reported first time from this plant[16,17].

Methicillin defiant *Staphylococcus aureus* (MRSA) and *Proteus vulgaris* was found more susceptible towards 8-methoxy-3′,5′7′-trihydroxyflavone. However, the reasons discussed for highest susceptibility of these strains is might because of H^+-ATPase-mediated proton pumping. Next to 8-methoxy-3′,5′7′-trihydroxyflavone, Galangin was also showed good anti-bacterial activities on MRSA strain. Our studies are quite different in source and type of the compound isolated even though antibacterial activity was reported for the leaf extracts. As per the present study it has been understood that compounds isolated are more effective in Gram positive organisms. Thus, our results correlated with literature reports that flavonoids are more effective against Gram negative strains and show selective inhibition of Gram positive strains. The ability of complex formation with soluble proteins and simultaneous disruption of cell wall are the major reasons discussed for significant anti-bacterial activity of the isolated compounds. Also anti-fungal activity noticed that Galangin was more effective against *Aspergillus niger* by producing highest inhibition zone. *Candida albacans* exhibited considerable susceptibility against tested compounds and showed resistant against 8-hydroxy-7,3′,4′,5′-tetramethoxy flavones. Thus, our studies evidently supports the anti-fungal activity of Galangin as because of its significant relation as antifungal agent in contrast to wide variety of fungi.

Compounds tested for scavenging of free radicals were proved as good hydrogen donors and showed direct inhibition of all free radicals tested (p<0.5). However, electron potential, number and position of hydroxyl groups substituted might be key factors and influence the variations in the activity. On the basis of numerous earlier and modern iresearch it is clear that flavonoids follow Bor's criteria to prove them as good anti-oxidants. According to this, flavonoids with CII-CIII

double bond and presence of 3-0H and 5-0H groups act as good anti-oxidative agents. Compounds isolated in the current study establish the same relation and exhibited structure-activity relationship for their attributed anti-oxidant activity. Galangin was found most effective in scavenging of free radicals. This might because of the ability of galangin in modulation of enzyme activity and inhibition of genotoxicity generated by chemicals.

Compounds significantly inhibited the process of egg albumin denaturation in concentration dependent manner and proved as good anti-inflammatory agents. However, group of flavonoids possess long lasting history as role in inhibition of inflammation process. Mechanism and action of these flavonoids includes specific inhibition of enzyme systems involved in the initiation of inflammatory processes.

Flavonoids are the family of substances with interesting biological properties which includes anti-microbial, anti-inflammatory, anti-cancer, anti-viral, immunomodulatory. As the literature deficient on pharmacological reports of heart wood of root and root bark, the investigations and results of current study could become the first report.

V. CONCLUSION

By the data obtained in the above study, it has been concluded that flavonoids and chalcones showed significant medicinal properties. Thus, these isolated compounds can be used for the action of various human alignments.

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