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NOTE

Antioxidant Activity of Flavonoid Isolated from *Meyna spinosa* Leaves

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A flavonoid was isolated first time from the leaves of *Meyna spinosa* (Rubiaceae). Antioxidant study revealed that the isolated flavonoid possess strong antioxidant activity. The isolated compound at 20 µg/mL concentration possesses 42.92 % DPPH$^{\bullet}$ and 40.92 % NO$^{\bullet}$ scavenging effect, respectively. Thus the isolated flavonoid could be a future for drug molecule in the treatment of various oxidative stress related diseases.

Keywords: *Meyna spinosa*, Leaves, Flavonoid, Antioxidant.

Meyna spinosa Roxb. ex Link (family: Rubiaceae) is an important folk medicinal plant used by the ethnic people of North-East India. Fruits and barks of the plant are used to treat headache, while the fruits and leaves of the plant are beneficial in diabetes, jaundice and other gastrointestinal disorders. Tender leaves, ripe fruits and seeds are beneficial to cure skin infections, pimples, indigestion and dyspepsia[1-3]. Recently, oleanolic acid were isolated from the fruits of *M. spinosa* which possess antimicrobial activity against *B. subtilis*, *K. pneuminiae*, *E. coli*, *S. aureus* and *C. albicans*[4]. In our recent investigation we reported leaves extracts of *M. spinosa* especially methanol extract possess significant free radical scavenging activity[2]. The present study was undertaken to isolate few chemical constituent of the plant with antioxidant activity.

Leaves of *Meyna spinosa* Roxb. were collected from Tripura, India and identified by Dr. B.K. Datta, Department of Botany, Tripura University, Tripura, India (TU/BOT/HEB/SS23072011a). Dried leaves of *M. spinosa* were pulverized and extracted with methanol using Soxhlet apparatus (yield 15.20 % w/w). Methanolic extract of *M. spinosa* leaves was further fractionated using petroleum ether, ethyl acetate and methanol in the order of increasing polarity. Preliminary study have showed that methanolic fraction of *M. spinosa* (MFMS) possess better antioxidant activity, therefore methanolic fraction of *M. spinosa* was further subfractionated to isolate pure compound using column chromatography (silica gel as stationary phase and chloroform as mobile phase). There were 11 bands distinctly visualized for methanolic fraction of *M. spinosa* which were collected serially. Isolated components of *M. spinosa* were named as recorded as MS-1 to MS-11.

Antioxidant activities of sub-fractions (MS-1 to MS-11) were evaluated using two methods. DPPH$^{\bullet}$ scavenging activity of subfractions (10 and 20 µg/mL) was measured in terms of hydrogen-donating or radical scavenging ability, using stable DPPH$^{\bullet}$ [5]. Nitric oxide radical (NO$^{\bullet}$) scavenging activity of subfractions (10 and 20 µg/mL) was also determined using Griess reagent as described by Yen *et al.*[6].

Based on antioxidant activity 4 sub fractions of *M. spinosa* were selected for further analysis. MS-9 produced highest DPPH$^{\bullet}$ scavenging activity followed by MS-7, MS-4, MS-10. While, MS-7 showed highest NO$^{\bullet}$ scavenging effect followed by MS-9, MS-10 and MS-4 (Table-1). In our previous investigation we have reported that methanolic extract of *M. spinosa* leaves possess significant antioxidant activity and contain significant amount of total phenolic (90.08 mg GAE/g of dry material) and total flavonoid compound (58.50 mg QE/g of dry material)[3]. In this study, subfractions showed potent scavenging effect, which implies that fractions contain some potent antioxidant components.

DPPH scavenging effect by antioxidant compound is based on the principle that antioxidants molecule reduces the stable DPPH$^{\bullet}$ to a yellow coloured diphenyl-picrylhydrazine[7]. MS-4, MS-7, MS-9, MS-10 have exhibited strong activity,

TABLE-1
FREE RADICAL SCAVENGING ACTIVITY OF SUBFRACTIONS OF *M. spinosa*

Fractions	Conc. (µg/mL)	Percentage inhibition (scavenging effect)	
		DPPH$^{\bullet}$	NO$^{\bullet}$
MS-1	10	14.42 ± 0.09	5.48 ± 0.09
	20	18.30 ± 0.12	9.04 ± 0.11
MS-2	10	05.95 ± 0.05	8.75 ± 0.09
	20	15.36 ± 0.18	13.93 ± 0.18
MS-3	10	20.95 ± 0.18	6.90 ± 0.09
	20	28.40 ± 0.26	13.48 ± 0.16
MS-4	10	33.99 ± 0.17	18.04 ± 0.17
	20	40.27 ± 0.22	29.26 ± 0.29
MS-5	10	7.11 ± 0.11	19.30 ± 0.23
	20	16.30 ± 0.28	24.03 ± 0.12
MS-6	10	19.18 ± 0.64	16.94 ± 0.14
	20	24.04 ± 0.15	24.49 ± 0.20
MS-7	10	32.02 ± 0.20	29.02 ± 0.20
	20	42.92 ± 0.30	40.92 ± 0.22
MS-8	10	8.97 ± 0.19	11.97 ± 0.17
	20	21.15 ± 0.42	19.01 ± 0.27
MS-9	10	38.52 ± 0.22	25.05 ± 0.20
	20	51.07 ± 0.34	34.17 ± 0.34
MS-10	10	29.08 ± 0.32	23.04 ± 0.22
	20	35.72 ± 0.40	32.50 ± 0.30
MS-11	10	22.22 ± 0.29	15.01 ± 0.17
	20	29.10 ± 0.32	21.13 ± 0.24
Ascorbic acid	20	93.11 ± 0.20	50.60 ± 0.21

which may be accredited to a direct role in trapping free radicals by donating hydrogen atom. NO$^{\bullet}$ is relatively stable but can react with superoxide which further provokes the generation of potent and toxic oxidant peroxynitrite[8,9]. Thus sub-fractions significantly scavenge NO$^{\bullet}$ which further expands their role as a potent antioxidant.

TLC was performed for selected *M. spinosa* fractions (MS-4, MS-7, MS-9, MS-10), but MS-7, MS-9, MS-10 showed single spot, which indicated that MS-7, MS-9, MS-10 were isolated as pure form, other fractions may contain a mixture of antioxidant constituents. Thus these three fractions were selected for spectral analysis.

Chemical and spectral analysis reveals the MS-7 is a flavonoid [2-(3-cyclopropyl-4-hydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl acetate] and the tentative structure of MS-7 is given in Fig. 1. The flavonoid was isolated first time form *M. spinosa* leaves.

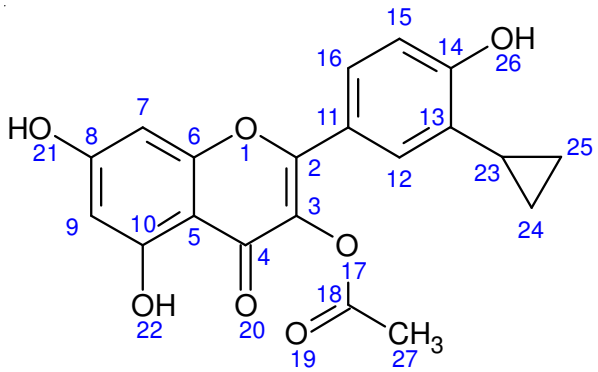

Fig. 1. Structure of MS 7

IR spectroscopic data showed peak at 3685 cm^{-1} (OH str), 3019.42 cm^{-1} (-CH str in $COCH_3$), 2400.42 cm^{-1} (-O-C-Ostr) 1720.28 cm^{-1}(C=Ostr in 6 member ring), 1602.39 cm^{-1} (Ar skeletal vibration), 1425.04 cm^{-1} (Phenolic OH), 1215.62 cm^{-1} (C-O str for fused diaryl system), 1078.53 cm^{-1} (C-Ostr in cyclic 6 member ring), 1017.73 cm^{-1} (cyclopropane skeletal system). ^{1}H NMR spectroscopic data showed peak at δ 4.823 (9H), 4.545(15 H), 3.808 (16 H), 3.779 (7H), 3.757 (12H), 3.684 (21H), 3.641 (26H), 3.627 (22H), 3.614 (23H), 3.601 (24H), 3.300 (25H), 1.282 (27H). ^{13}C NMR spectroscopic data showed peak at δ 74.47 (8C, 10C, 14C), 73.17 (4C), 73.60 (9C), 71.54 (2C, 3C), 65.28 (7C), 49.79 (5C, 6C), 49.58 (15C, 16C), 49.36 (11C), 49.15 (12C), 48.94 (18C), 48.51 (23C, 24C, 25C), 48.73 (13C), 30.88 (27C). Mass spectroscopy data showed molecular weight peak at *m/z* 368 appeared as (M-1)$^+$. Base peak appeared as (M+1) for $[C_{18}O_{14}O_5]^+$.

Spectral analysis of MS-9 and MS-10 was also carried out. Mass spectra of both fraction showed same base peak as like MS-7, but molecular ion peak is different, which conclude that both are available in mixed form; hence could not be separated.

Antioxidant compounds thus can play an important role in prevention/treatment of oxidative stress induced diseases. Flavonoids are the large group of ubiquitous molecules and most important class of polyphenolic compounds. Their planar structure, number and position of their hydroxyl groups as well as the presence of the C2-C3 double bond are vital for their scavenging effect. Several investigators have reported their metal chelating effect, free radical producing enzymes preventive capacities also[10].

In conclusion, the isolated flavonoid showed potent antioxidant activities which could be a future drug molecule in the treatment of various oxidative stress related diseases. Further study will be useful to isolate the other individual components present in the plant.

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