ORIGINAL RESEARCH PAPER

Biological Science

HRLCMS ANALYSIS OF MORUS NIGRA L. AND CISSUS QUADRANGULARIS L.FOR IDENTIFICATION OF ANTIOXIDANT COMPOUNDS

KEY WORDS: DPPH, Antioxidant, Morus nigra and Cissus quadrangularis, ascorbic acid.

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ABSTRACT

The antioxidant activity of leaves extracts of Morus nigra and Cissus quadrangularis was evaluated by free radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazil (DPPH), reducing power assay. The results of the assay were then compared with a natural antioxidant ascorbic acid (vitamin C). The Methanolic extract of the Leaves of Morus nigra and Cissus quadrangularis is a potent source of compounds with antioxidant properties while the extract also exhibited significant free radical scavenging activity. The methanolic extract of Morus nigra and Cissus quadrangularis was subjected to preliminary phytochemical studies. The results indicate the presence of alkaloids, flavonoids, terpenoid proteins, and carbohydrates.

INTRODUCTION

Reactive oxygen species and free radicals play an important role in the initiation and evolution of numerous diseases. The use of compounds with antioxidant property is expected to be useful for the treatment of these diseases. Therefore, there has been a growing interest in finding novel antioxidants in order to meet the requirements of pharmaceutical industries1. From classical period humankind has always been interested in naturally occurring components from plants and animals sources. Various extracts of different plant parts have been widely used in folk medicines and perfumes as well as in food flavor, preservatives and preparation and are more commonly utilized in chronic as well as common diseases. Plants contain several active compounds known as bioactive compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids that are deposited in their different parts.

MATERIAL AND METHODS

PLANT MATERIAL

Disease free leaves were collected and identified by following the flora of Marathwada by V.N.Naik. The collected leaves were surface sterilized with 0.1% mercuric chloride & then washed with D/W 2-3 times separately & shade dried. Fine powder were made after complete drying and used for the experimental work.

SOLVENT EXTRACTION OF LEAVES

Extracts were made in 80% methanol at room temperature by simple extraction method (Deshpande et al). 10 gm dried powder of leaves mixed with 100ml solvent in 250 ml flask and were kept on shaker for 24 hrs. Then it was allowed to stand for the 30 min to stand the plant material. Thereafter it was filtered & centrifuged at 5000 rpm for 15 min .The supernatant was collected &solvent was evaporated at 45 0C in rotary evaporator to make the final volume 1/5 of the original volume.

THIN LAYER CHROMATOGRAPHY-

TLC is the most commonly used planar chromatographic method in natural product research. This is the easiest and cheapest technique and can be applied in the analysis, isolation and setting the parameters for column chromatography [9]. Usually, silica or alumina (more polar) is used as the stationary phase and organic solvents (less polar) are used as the mobile phase. This situation is categorized as normal phase chromatography. In contrast to this, reverse phase TLC is available, in which stationary phase is alkyl bonded silica or alumina (less polar) and mobile phase is polar solvent like water, alcohol etc.

DPPH FREE RADICAL SCAVENGING ACTIVITY –

The free radical scavenging activity was followed by the DPPH method. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (50-250 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (50 to250 g/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = [(A control- A test) / A control] × 100

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the methanolic leaves extract was expressed as IC50 and compared with standard. The IC50 value was defined as the concentration (in g/ml) of extracts .That scavenges the DPPH radicals by 50%.

HRLC-MS ANALYSIS: At SAIF (Sophisticated Analytical Instrument Facility), IIT Powai, Mumbai, equipment and conditions Identification of metabolites from an active sub-fraction of methanol extract was carried out. Samples were analyzed on a LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 min. then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 min. The MS analysis was carried out by ESI positive ionization mode. HRLC-MS gives the presence of fatty acids, organic compounds, phenolics, alkaloids, phytoharmone, coenzyme, aminopyrimidines, dipeptide and tripeptides like important metabolites in leaves. For HRLC the Column used---zorbax eclipse c18,2.1 x 150mm 5-micron.

RESULTS –

DPPH free radical has strong electron attracting ability from antioxidant. The graph showed that the conc. of methanolic extract is directly proportional to Absorbance. The Maximum scavenging activity is shown at the lowest conc.200µl/ml which was 3.50 %. The order of five scavenging activity is 50>100>150>200<250 µl/ml. (Table1.) The percentage of scavenging activity for methanolic extract of Morus nigra and Cissus quadrangularis L. leaves is inversely proportional to absorbance .Ascorbic acid was used as control. The TLC analyzed result of methanolic extract of Morus nigra and Cissus quadrangularis L with mobile phase Chloroform: Methanol: ethyl acetate (8.0:1.5:1.0).Mean of the Rf values of TLC analysis is 0.57.The HRLCMS results also revealed that the presence of antioxidant like Carnitine, Racepinephrine ,Swetenine, Apiin is a natural flavonoid, a diglycoside of the flavone apigenin .Vigabatrin also present in methanolic

extract and posses antioxiant and anti-epileptic property as gabapentin .The anti-epileptic drug vigabatrin was developed as an inhibitor of gamma-aminobutyric acid transaminase, and its ability to increase inhibition in the central nervous system led to its testing in an animal model. In animal models chronic use of vigabatrin is associated with irreversible myelin vacuolation. Antioxidant drugs change the antioxidant capacity of the body. Oxidative stress of the body increased when valproic acid and carbamazepine were used chronically (Miijga n Cengiz et al , (2005). probenecid were highly effective at reducing oxidative stress detected by dichlorofluorescein fluorescence. Lina Du et al (2016).

DISCUSSION -

Antioxidant assay revealed that the free radical scavenging activity showed by extract at different concentration. The maximum %scavenging activity at 250 µl/ml for both the plant extract was 72 % and 60 % and minimum activity at 50 µl/ml was 56 % and 48 % which was nearer to the standard activity of ascorbic acid. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Table 1 shows the percentage of DPPH radical scavenged by ascorbic acid and methanolic extract of leaves at various concentrations (g/ml). A substance may act as an antioxidant due to its ability to reduce reactive oxygen species by donating hydrogen atom.

The reducing property of methanolic leaves extract of Morus nigra and Cissus quadrangularis L. implies that it is capable of donating hydrogen atom in a dose dependent manner. The high content of phenolic compounds in the extract may be a contributing factor towards antioxidant activity because the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which can function as hydrogen donor. Research in the direction of partial isolation and characterization of the constituents of methanolic leaves extract of Morus nigra and Cissus quadrangularis L. in order to decipher the specific phytochemical constituent(s) responsible for the free radical scavenging activity of the plant. When this is done, extracts of Morus nigra and Cissus quadrangularis L, L. could find important application in phytotherapy. Qualitative

Compound Report

Data File	M-Nigra YAP4.d	**Sample Name**	M-Nigra YAP4
Sample Type	Sample	**Position**	P1-D7
Instrument Name	Instrument 1	**User Name**	
Acq Method	30mins_+ESI_10032014_MSMS.m	**Acquired Time**	1/28/2017 6:01:37 AM
IRM Calibration Status Comment	Success	**DA Method**	default.m

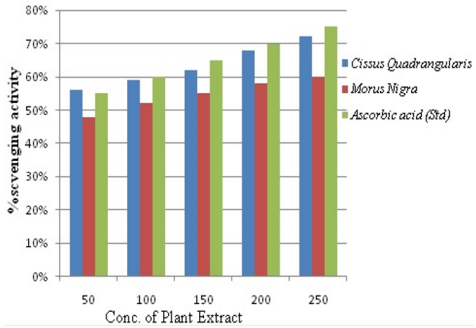
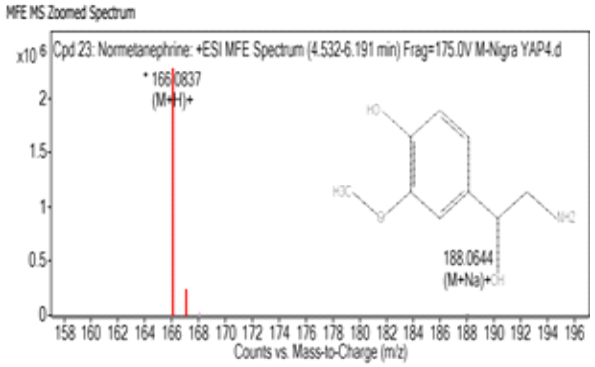


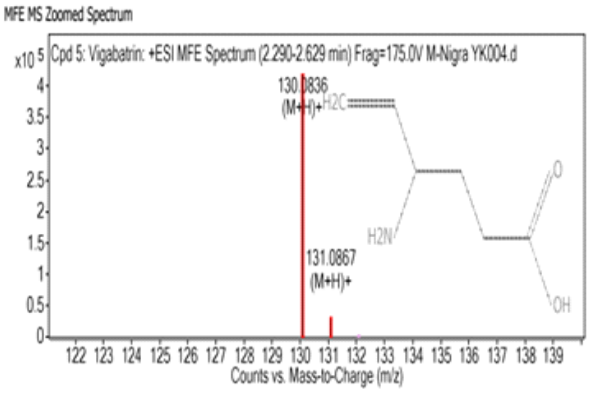
Table 1- Scavenging activity of MeoH leaf extracts

Sr.no.	Conc. of extract	Percent scavenging activity		
		Morus Nigra	Cissus Quadrangularis	Ascorbic acid (Std)
1	50	48%	56%	55%
2	100	52%	59%	60%
3	150	55%	62%	65%
4	200	58%	68%	70%
5	250	60%	72%	75%

Choromatograms of *Morus nigra* L. through HR-LCMS analysis.



MS Compound Spectrum With Structure



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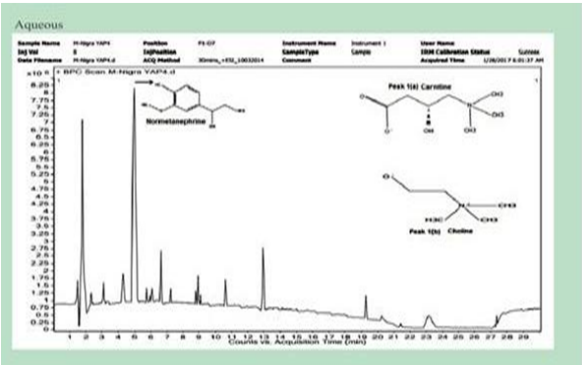
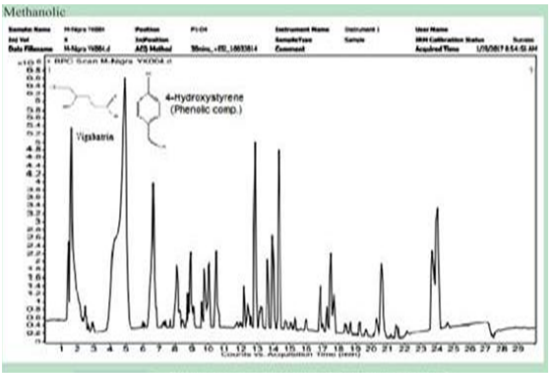




RT	Mass	Name	Formula	DB Diff (ppm)
1.677	104.1092	Choline	C5 H14 N O	16.35
1.776	133.0741	1,4-Dideoxy-1,4-Imino-D-Arabinitol	C5 H11 N O3	1.58
1.823	162.1108	Carnitine	C7 H16 N O3	13.68
4.327	145.1101	2S-aminoheptanoic acid	C7 H15 N O2	1.38
6.63	183.089	Racepinephrine	C9 H13 N O3	2.84
9.095	186.1612	4-methyl-decanoic acid	C11 H22 O2	4.4
12.959	563.3116	dihydroergocornine	C31 H41 N5 O5	1.54
12.96	568.267	Swietenine	C32 H40 O9	0.39
19.267	452.2932	Dihydrocelastrol	C29 H40 O4	1.09

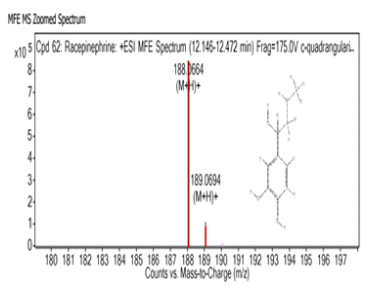
RT	Mass	Name	Formula	DB Diff (ppm)
1.833	133.0728	1,4-Dideoxy-1,4-Imino-D- Arabinitol	C5 H11 N O3	8.58
2.428	129.0763	Vigabatrin	C6 H11 N O2	10.46
2.668	285.1016	Probenecid	C13 H19 N O4 S	6.5
4.895	120.0585	4-Hydroxystyrene	C8 H8 O	8.03
6.628	183.0874	Racepinephrine	C9 H13 N O3	11.67
6.633	113.0836	epsilon-Caprolactam	C6 H11 N O	3.88
6.75	141.0778	Ethosuximide	C7 H11 N O2	8.31
10.149	513.2695	Sulfolithocholylglycine	C26 H43 N O7 S	12.75
10.491	141.0778	Ethosuximide	C7 H11 N O2	8.17
10.492	113.0834	epsilon-Caprolactam	C6 H11 N O	5.57
10.567	200.0771	Barbituric acid, 5-ethyl-5-(2-hydroxyethyl)-	C8 H12 N2 O4	13.24
10.568	94.0076	Dimethyl sulfone	C2 H6 O2 S	13.4
11.278	395.2027	Spiperone	C23 H26 F N3 O2	4.5
11.715	186.1599	3-methyl-decanoic acid	C11 H22 O2	11.37
12.874	563.3085	dihydroergocornine	C31 H41 N5 O5	4.05
12.875	568.2636	Swietenine	C32 H40 O9	6.39
13.965	326.203	Hydroquinine	C20 H26 N2 O2	10.81
19.255	452.2904	Dihydrocelastrol	C29 H40 O4	5.03
25.233	586.2709	Khivorin	C32 H42 O10	11.82

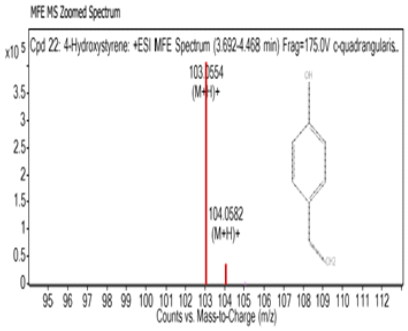
2.241	116.0581	I^-Diacetylhydrazine	C4 H8 N2 O2	4.15
2.786	116.0832	2-methyl valeric acid	C6 H12 O2	4.17
5.066	138.0891	nn-Dimethylaniline-n-oxide	C8 H12 N O	20.48
6.701	183.0876	Racepinephrine	C9 H13 N O3	10.41
9.287	188.1162	Acetyllysine	C8 H16 N2 O3	0.47
10.265	159.1246	DL-2-Aminooctanoic acid	C8 H17 N O2	8.36
12.875	563.309	Dihydroergocornine	C31 H41 N5 O5	3.22
12.876	568.2643	Swietenine	C32 H40 O9	5.1
19.267	452.2909	Dihydrocelastrol	C29 H40 O4	3.98

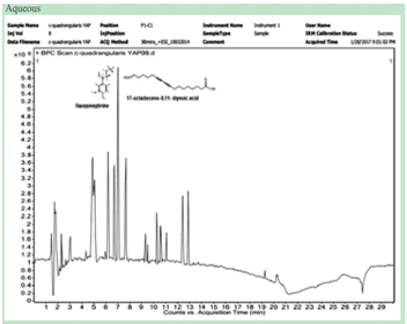
2.671	285.102	Probenecid	C13 H19 N O4 S	5.15
4.288	120.0586	4-Hydroxystyrene	C8 H8 O	9.32
6.607	113.0838	epsilon-Caprolactam	C6 H11 N O	2.52
6.612	121.0881	Phenylethylamine	C8 H11 N	8.46
6.614	183.0878	Racepinephrine	C9 H13 N O3	9.63
6.734	141.0781	Ethosuximide	C7 H11 N O2	6.23
7.159	418.1765	Nimodipine	C21 H26 N2 O7	5.86
7.576	121.0881	Phenylethylamine	C8 H11 N	9.02
7.665	164.0935	Isonicotinamide, 2-propyl-	C9 H12 N2 O	8.66
7.914	564.1392	Apiin	C26 H28 O14	15.4
8.279	188.116	Acetyllysine	C8 H16 N2 O3	0.62
8.28	128.1193	Octanal	C8 H16 O	6.23
10.535	244.0799	5-azacytidine	C8 H12 N4 O5	3.47
12.861	563.309	dihydroergocornine	C31 H41 N5 O5	3.16
12.862	568.264	Swietenine	C32 H40 O9	5.69
15.946	408.2411	Dextromoramide M2	C25 H32 N2 O3	0.48
16.035	444.1975	Mitoxantrone	C22 H28 N4 O6	7.6
19.258	452.2908	Dihydrocelastrol	C29 H40 O4	4.13
20.71	286.2475	Avocadene	C17 H34 O3	11.45

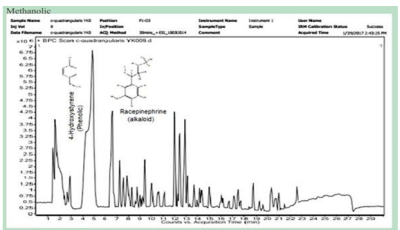
CONCLUSION

Oxidative stress can arise from overproduction of ROS by metabolic reactions that use oxygen and shift the balance between oxidant/antioxidant statuses in favor of the oxidants. ROS are produced by cellular metabolic activities and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules because of unpaired electrons in their structure and react with several biological macromolecules in cell, such as carbohydrates, nucleic acids, lipids, and proteins, and alter their functions. ROS also affects the expression of several genes by upregulation of redox-sensitive transcription factors and chromatin remodeling via alteration in histone acetylation/deacetylation. Regulation of redox state is critical for cell viability, activation, proliferation, and organ function. Experiments confirming these activities of the extract of Morus nigra and Cissus quadrangularis in an in vivo system would be necessary. However, herbal remedies often do not produce any side effects. Therefore, alternative medicine become popular remedy to various types of ailments In conclusion, Morus nigra and Cissus quadrangularis extracts have revealed significant antibacterial activities against test organisms used for the study.









Choromatograms of *Cissus quadrangularis* L. through HR-LCMS analysis.

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