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EVALUATION OF FREE RADICAL SCAVENGING POTENTIAL OF 7-HYDROXY–4-METHYLCOUMARIN ISOLATED FROM MIMOSA NILOTICA (L)

Pharmacology

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ABSTRACT

Introduction: Medicinal plants have been used for the treatment of diseases all over the world before the advent of artificial clinical drugs and are known to contain substances that can be used for therapeutic uses. Antioxidations act asreducing agents, free radical scavengers, quenchers of singlet oxygen molecule, and activators for antioxidative enzyme to reduce the damage caused by free radicals in biological pattern. **Materials and Methods:**The isolated organic compound from Mimosa nilotica was subjected to column chromatographic separation and was characterized by UV, IR, ^{1}H-NMR and ^{13}C-NMR.DPPH assay was conducted to assess the effect of the ethanolic extract and 7-hydroxy–4-methylcoumarin. **Result and Discussion:**The reports of the in-vitro antioxidation assay indicated that the ethanol extract was more potent than 7-hydroxy–4-methylcoumarin. 7-hydroxy–4-methylcoumarin and ethanolic extractexpress excellent free radical scavenging activity. From the reports it is proved that the reducing power of bioactive constituent is associated with antioxidationpotential. Thus a correlation is proved between reducing power and the antioxidation effect. **Conclusion:**Previous reports are confirmed that the reducing power of bioactive constituents is associated with antioxidation potential. People who eat various parts of fruits and herbs have a lower risk of heart disease and some neurological diseases and there is evidence that some types of herbs, and fruits in general, protect against some malignant.

KEYWORDS

Antioxidaion potential, Phytoconstituent, Free radical, Mimosa nilotica.

INTRODUCTION

India has classical history of regular medicines, with two widely flourishing systems of treatments i.e. Ayurvedic and Unani systems[1]. Phyto-medicines are biologically active constituents which are enormously found in herbs, fruits, legumes, nuts, vegetables, whole grains, seeds, fungi and spices which gives health benefits for humans and cure many ailments, further they act as macronutrients and micronutrients[2]. Bioactive constituents are deposited in different parts of the plants, such as in roots, stems, leaves, flowers, fruits and seeds. They protect the plants from diseases and contribute color, odour and flavor. More than 4,000 bioactive constituents have been separated and benefited by the humans in all aspects[3]. Still research is on-going to isolate the bioactive constituents[4,5]. Phytochemists prepare the extracts from the plant materials and subject these extracts to biological screening in bioassays and commence the process of isolation and characterization of the active compounds through bioassay directed fractionations. A golden triangle consisting of ayurveda, modern medicine and science.

The bark of Mimosa nilotica(Family: Mimosaceae)). commonly known as black babool or babul or babbula, has been traditionally used as an astringent, acrid, cooling, styptic, emollient, anthelmintic, constipating, depurative, aphrodisiac, diuretic, expectorant, emetic and nutritive agent. It is useful in vitiated conditions of kapha and pitta, haemorrhages, wounds, ulcers, helminthiasis, ascites, chronic dysentery, diarrhoea, leprosy, leucoderma, skin diseases, burning sensation, cough, bronchitis, leucorrhoea, seminal weakness, oral ulcers and odontopathy. There are many reports indicating a variety of activities for Mimosa nilotica bark. The acetone extract of the bark has been reported to exhibit antimutagenic and cytotoxic activities[6] It is rich in tannins and the tannin content of the bark varies from 12-20%. Several polyphenolic compounds have been reported to be present in the bark such as,catechin,gallic acid epicatechin umbelliferone, ,dicatechin, quercetin, and leucocynidin gallate.

Babul is a medium sized, thorny, nearly evergreen tree that can reach a height of 20-25 m but may remain a shrub in poor growing conditions. The trunk is short, thick and cylindrical, covered with grey bark. The crown may be flattened or rounded. The root system depends on the growing conditions and subspecies: a deep taproot in dry conditions and extensive lateral roots in flooded conditions. The leaves are elliptical, grey-green, hairy leaflets. Flowers are sweetly scented and bright to golden yellow in colour. The fruits are linear, flattened, narrow indehiscent pods, dark-brown to grey in colour and glabrous or velvety. The pods are elliptical, flattened, bean-shaped dark. It is widely used in ethno-medicine.

Antioxidations(antioxidants) are molecules capable of decreasing or preventing oxidation reaction in the cells of the substrate molecules. Free radicals are produced by oxidation reactions, which lead to a chain reaction and subsequently cause number of diseases in humans. Antioxidation compounds are capable of suppressing or terminating these chain reactions by removing free radical intermediates and other oxidation reactions[7]. The harmful effect of these free radicals can however be overcome by antioxidant substances. There is a good progress in the research regarding natural antioxidants from plants to be safe and alternate to synthetic antioxidants .Many plant-based compounds such as ascorbic acid, vitamin E, polyphenols, xanthophylls, carotenesare have been investigated for their antioxidation potential in different food products to improve their shelf life and functional properties[8].

Bioorganic molecules can be oxidized by free radicals. This oxidative damage has an important etiological role in aging and the development of diseases like cancer, atherosclerosis, and other inflammatory disorders,cancer, neurological diseases, hyper tension etc.Artificial antioxidations, such as butylated hydroxyl anisole, are good free radical scavengers; however, they can be carcinogenic. Therefore, there is an increasing interest in searching for antioxidations of natural origin. Mimosanilotica has a versatile medicinal value as folk medicine. Antioxidations defense both enzymatic and non-enzymatic reactions to protect the body against oxidative damage. Non-enzymatic antioxidants are frequently added to the food to prevent lipid oxidation[9]. Natural antioxidation in plants are in the form of phenolic compounds such as phenolic acids, flavonoids, tocopherols etc. Biological activities of phenolic compounds involve free radical scavenging in cells. Phenols are found in the plant kingdom. The antioxidation activity of phenol is mainly due to their redox properties, hydrogen donor and singlet oxygen quenchers. Some phenols are proved to have hypotensive effects and antioxidant properties. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[10]. Hence we are planning to isolate the phytochemical from the ethanol extract of Mimosa nilotica bark and the isolated phytochemical was characterized by UV, IR, ^{1}H- NMR ^{13}C - NMR and its assessment of Free radical scavenging potential.

MATERIALS AND METHODS

Plant materials

The Mimosa nilotica bark was collected from Jayankondam dry land, Tamil Nadu state, India. They were identified and authenticated by Dr.L.Mullainathan, Professor in Botany of the Department of Botany, Annamalai University, Chidambaram, India.

General Experimental Procedures

Structural identification of the isolated compound is confirmed by the ^{1}H (DMSO d6, 300 MHz) and ^{13}C- NMR (100 MHZ, DMSO-d6), spectral data were recorded on a Bruker AMX 300 NMR spectrometer. IR spectrum was recorded with a Perkin Elmer RXI FT-IR spectrometer as a thin film on KBr plate. UV spectral data was recorded using UV- Visible Spectrophotometer Lambda 35 from Perkin Elmer.

Extraction and Isolation

The shade dried Mimosa nilotica bark (1000 gm) was ground to a fine powder and extracted with EtOH at 50°C. The extractions was collected as ethonolic extract and the solvent recovered by simple distillation. The resulting lower layer of the ethanolic extract was subjected silica gel column (60–120 mesh) using as a toluene/chloroform/acetone 40:25:25 as eluent. At regular interval, the eluents (each of ten ml) were collected and the progress of separation was identified by thin layer chromatography (TLC) (silica gel G 60 F254 TLC plates of E. Merck, layer thickness 0.2mm) using solvent system benzene/ethyl ether 1:1) and iodine vapour as detecting agent. Which indicated single spot on TLC (benzene/acetone 85:15, R_f 0.35) afforded the compound. The fraction was evaporated to dryness and crystallization using ethylacetate produced the white powder, melting point 190°C [11].

Structural elucidation

Structural elucidation of the isolated compound from ethanol extract of Mimosa nilotica bark were recorded by UV, IR, ^{1}HNMR and ^{13}C-NMR spectroscopic methods. From the Previous report, melting point and spectral data, the isolated compound was confirmed as 7-hydroxy–4-methylcoumarin (**$C_{10}H_{18}0_3$**).

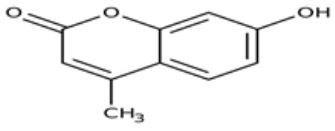

Figure-1: Structure of 7-hydroxy–4-methylcoumarin

Statistical Analysis

Assessment of statistical significance was expressed as mean± SD.

Evaluation of In-Vitro Free Radical Scavenging Potential DPPH Scavenging Potential Principle

Antioxidations react with DPPH (*2, 2-Diphenyl-1-Picrylhydrazyl*), which is a stable free radical and is reduced to the DPPHH and as a consequence the absorbance decreases.

(DPPH) + (H-A) → **DPPH-H + (A)**
(Purple) (Yellow)

Procedure

About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1mL of this solution was added to 3 mL of the fraction dissolved in ethanol at different concentrations (25-400 µg/mL).The well shaken mixture was allowed to stand at 25°C for 30 minutes and the absorbance was recorded at 517 nm using a spectrophotometer. Reduce the absorbance of the reaction mixture indicates higher antioxidation. The capability to scavenge the DPPH radical was calculated using the following equation.

$$DPPH\ Scavenged\ (\%) = \left[\frac{A_{control} - A_{test}}{A_{control}} \right] \times 100$$

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 % of scavenging potential at different concentrations was determined and the IC_{50} value of the fractions was compared with that of vitamin-C which was used as the standard[12].

RESULT AND DISCUSSION

Structure of 7-hydroxy–4-methylcoumarin

The isolation of Compound from ethanol extract of Mimosa nilotica bark was subjected to column chromatographic separation analysis. Its UV spectrum in neutral (EtOH) displayed an absorption bands at 267.06 (- C=O) nm and 318.342 nm (-OH). Compound exhibited UV absorption bands at 330 nm indicating Coumarin derivative(Table-1). The IR absorption showed the absorption bands of hydroxyl (-OH) at 3080.42 cm^{-1}, aryl ketonic stretch (-C=O) stretching at 1684.20cm^{-1} and C-O stretch of phenol at 974.71 cm^{-1}(Table-2). The ^{1}H-NMR spectrum showed four signals in the aromatic region. AB type doublet of α, β- olefinic proton at δ 6.14 (H-3) .The tri substituted benzene ring was proposed from an ABX signal of aromatic protons H-5, H-6 and H-8 at δ 7.58 (H-5), δ 6.70(H-6) and δ 6.78 (H-8) respectively(Table-3).One singlet signal at δ 2.367 indicating methyl group. The decoupled ^{13}C-NMR spectrum revealed ten signals indicating presence of ten types of carbons. ^{13}C-NMR (DMSOd6, 100 MHz): spectrum shows five signals at δ 101.40 (C-8), 111.90 (C-6), 112.73 (C-3), 126.36 (C-5) 154.30 (C-4) and shows the presence of four quaternary carbons. One signal at δ 160.06 (C-2) was described to carbonyl function (C-2) of Coumarin derivative. Another downfield intense signal at δ 160.21 (C-7) was indicative of hydroxyl substitution at C-7 position and two others were δ 110.17(C-10) and δ 155.74 (C-9). One signal at δ 18.98 (C-10) was described to substituted methyl group (Table-3). The position of the absorption bands and the shape of the spectra were in complete resemblance with Umbelliferone derivative[13].The isolated compound was confirmed as 7-hydroxy–4-methylcoumarin or Hymecromone or 4-Methyl umbelliferone (Figure-1).

A large number of medicinal plants containing flavonoids, saponins and polyphenolic components have been reported to exhibit a strong potential of antioxidant activity. It is known that free radicals play a definite and important role in immune system physiology; hence the antioxidation potential of the bark was explored. Assays was conducted to assess the effect of the ethanolic extract and 7-hydroxy–4-methylcoumarin on free radicals like DPPH(Table-4). The results of the in vitro antioxidant assay indicated that the ethanol extract was more potent than 7-hydroxy–4-methylcoumarin. DPPH radical scavenging activity has been widely used to evaluate the antioxidation potential of the plant extracts and diets[14]. This suggests that the barks contain more phenolics that may be attributed to the antioxidant properties of Mimosa nilotica. This is the first report of the isolation and antioxidant potential of 7-hydroxy–4-methylcoumarin from Mimosa nilotica[15]. Literature reports are proved that the reducing capacity of bioactive constituents is correlated with antioxidation potential. Thus a correlation is evidence between reducing power and the antioxidant effect[16].

Table-1: UV- spectrum of 7-hydroxy–4-methylcoumarin

7-hydroxy–4-methylcoumarin	
λmax(KBr)in nm	**Chromophore Type**
267.06	C=O aryl ketone
318.342	O-H of phenol

Table-2 IR (KBr) spectrum of 7-hydroxy–4-methylcoumarin

7-hydroxy–4-methylcoumarin	
Peak Position (in cm^{-1})	**Interatomic Bond**
3080.42	O-H stretching vibration of phenol
1684.20	C=O aryl ketonic stretch
1603.77	C-C aromatic ring stretch
1382.55	O-H bending of phenol
1235.45	C-H bond in aromatic hydrocarbon
974.71	C-O stretch of phenol
798.53,645.68	C-H bending of aromatic hydrocarbon

Table -3: NMR spectrum ((δ in ppm) of 7-hydroxy–4-methylcoumarin

Position	^{1}H-NMR	^{13}C-NMR
1	-	160.06
2	-	112.73
3	6.14(s, 1H)	155.73
4	2.34 (s, 3H,-CH3)	126.36
5	7.58-7.60(d, 1H, Ar-H)	111.90
6	6.70(d, 1H, Ar-H)	160.21
7	-	101.40
8	6.78-6.81 (m, 1H, Ar-H),	154.74
9	-	110.17
10	-	110.17
11	-	18.98

Table-4: DPPH radical scavenging activity of 7-hydroxy–4-methylcoumarin(HMC) and ethanolic bark extract of Mimosa nilotica (EMN).

Concentration(µg/mL)	% Percentage of Inhibition		
	AA	HMC	EMN
0.5	39	26	38
1.0	42	38	42
1.5	50	50	52
2.0	56	56	59
IC50 (µg)	0.298	0.486	0.275

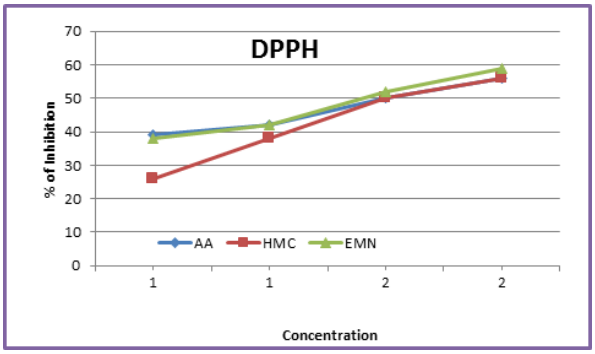

Figure 2: DPPH radical scavenging activity of Ascorbic acid (AA) 7-hydroxy–4-methylcoumarin(HMC) and ethanolic bark extract of Mimosa nilotica (EMN

CONCLUSION

This study showed that the methanol and aqueous extracts of the barks (Mimosa nilotica) exhibited potent antioxidant activity. The presence of high levels of phenolic compounds in the extracts may have partly contributed to the observed antioxidant activities. This study provides evidence on the potential health benefits of the pods of Mimosa nilotica. These barks on appropriate formulation in to dosage forms can serve as a rich source of phenolic compounds. Dietary sources, including plants, herbs, spices, vitamins and herbal extracts, play an important role in this regard.

CONFLICTS OF INTEREST

There are no conflicts of interest

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