Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(2): 2115-2119
Received: 10-01-2020
Accepted: 12-02-2020

Madhuri Vajha
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

Corresponding Author:
Madhuri Vajha
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

Bioprospecting of *Xylia xylocarpa* and its phytoconstituents

Madhuri Vajha

Abstract
Bioprospecting is the exploration, extraction and screening of biological diversity and indigenous knowledge for commercially valuable genetic and biochemical resources and also for potential use as pharmaceutical agents. This will probably lead to many new disease-fighting drugs, and leads to substantial profits for the pharma company. The research area of drug design and discovery is at a crossroads. *Xylia xylocarpa* or *Mimosa xylocarpa* (Mimosaceae) is a medium sized deciduous tree is selected for research study. As good anti-oxidant activity and good source of polyphenols, flavonoids and saponins, this study was promoted as part of screening new source of natural inhibitors of tyrosinase and 5-lipoxygenase activities and phytochemical composition with major marker compounds through bio-guided fractionation studies. This write-up provides schematic procedure for any natural product which shows potent pharmacological activity that can be used as alternative medicine. All compounds discussed in this report are presented here for the first time from this plant.

Keywords: Xylia, 5-lipoxygenase, flavonoids

1. Introduction
Natural products have been receiving a much demand due to its safety in addition to therapeutic effect and hence screening process has been accelerated to find out more potent and safe candidates for lifestyle disorders and skincare products. The plants of Mimosaceae family are used as food, medicinal, colouring, perfumery, construction (wood) material. Mimosaceae plants possess higher content of phenolic and flavanoid compounds [1]. *Xylia xylocarpa* or *Mimosa xylocarpa* is a medium sized deciduous tree usually characterized by bipinnate compound leaves and flowers are dominated by stamens. Its wood pulp is used for wrapping paper and timber is very hard used in constructions. The fruits and seeds are edible and a good source of protein. The ethanolic extract of bark, stem and leaves of *Xylia xylocarpa* showed potent DPPH radical scavenging activity [2]. As good anti-oxidant activity and good source of polyphenols, flavonoids and saponins, this study was promoted as part of screening new source of natural inhibitors of tyrosinase and 5-lipoxygenase activities and phytochemical composition with major marker compounds through bio-guided fractionation studies.
Arachidonic acid plays an important role in the process of inflammation which involves series of reactions. Prostagladins are responsible for pain [3]. Stimulation of neutrophils initiated cleavage of arachidonic acid from membrane phospholipids. Arachidonic acid is converted into leukotrienes by 5-lipoxygenase (5-LOX) and prostaglandins and thromboxanes by cyclooxygenase (COX) pathways respectively [4].
Literature revealed that the essential oils of myrtle and eucalyptus attenuated leucocyte activation by scavenging hydroxyl radicals indirectly produced by leucocyte degranulation there by interfering with inflammatory process by acting as antioxidants [5]. Therefore, the study of antioxidant property (DPPH) also provides some information about inflammatory process. Higher content of polyphenolic compounds possess high free radical scavenging activity (antioxidant) and anti-inflammatory activities [6], indicating a positive correlation between antioxidant activity and phenoloic compounds.

2. Materials & Methods
2.1 Plant material
The leaf of the plant *Xylia xylocarpa* was collected from surrounding of Vijayawada and identified and voucher specimen was deposited at Taxonomy division, Krishna University, Machilipatnam.

2.2 Extraction and bio-assay fractionation

The shade dried leaves were pulverized to fine powder. About 500 mg of powdered leaf was extracted with four volumes of 90% methanol repeatedly for four times. Total extract was combined and fine filtered and one sixth of part was directly concentrated to dry powder in rotary evaporator and made powder for phytochemical and bioactivity studies. The balance extract was concentrated to aqueous stage in rotary evaporator and was extracted with chloroform followed by ethyl acetate in twice the volume of the extract. Both chloroform and ethyl acetate soluble parts was concentrated separately in rotary evaporator and dried to powder under vacuum oven at 70 ^{0}C. Both chloroform and ethyl acetate soluble samples were given for bioactivity studies. Solvent residues of aqueous soluble part was distilled of and filtered. This filtered solution was passed through reverse phase adsorbent resin and fraction of water wash and methanol elution was collected. These fractions were concentrated to dry powder under vacuum and given for bio-activity study.

As ethyl acetate extract gave good inhibitory activity of tyrosinase, 5-liooxygenase and DPPH, was adsorbed in silica gel and flash column was run with hexane and ethyl acetate in increasing polarity. Those collected fractions were segregated by TLC pattern and similar fractions were mixed and concentrated to thick paste and again given for bioactivity studies. The fractions eluted with 30%acetone in chloroform showed better inhibitory activities among other fractions, this fraction was further sub-fractioned through silica gel flash column with elution of ethyl acetate: chloroform in increasing polarity. Again similar fractions on the basis of TLC pattern were segregated and concentrated to thick paste and given for bioactivity. The fractions eluted with 10% & 50% acetone in chloroform gave two pure known compounds i.e., methyl gallate and myricitrin respectively. These compounds were also submitted for bioactivity.

2.3 Determination of Total polyphenols

Total poly phenol percentage was determined by using the Folin-Ciocalteu reagent and expressed in gallic acid equivalents (GAE) in percentage. About 50mg of sample heated in 80ml of distilled water at 75 ^{0}C for ten minutes. The contents were cooled and made up to the volume of 100ml with distilled water and were filtered through Whatman No.41 filter paper. About 5 ml of the resulted solution was diluted to 25 ml with distilled water. About 2 ml of each standard (gallic acid) was pippted out in two 25 ml volumetric flasks and 10 ml distilled water and 2ml diluted phenol reagent to each volumetric flasks. The total volumes of the two volumetric flasks were made up with 29% w/v aqueous sodium carbonate solution. Two volumetric flasks were kept in dark place for 30 minutes. Blank was prepared similarly. The absorbance of samples against blank at 760 nm.

2.4 Determination of total flavonoids

Total flavonoids content was estimated by Aluminium chloride solution. Briefly 0.2 ml of standard solution (Rutin-flavonoid) in 1 ml of Aluminium chloride reagent was added and then made up to 10 ml with methanol. A standard blank was made. The absorbance of samples and standard was measured against reagent blank at 410nm. Total flavonoid content was expressed in percentage with respect to Rutin.

2.5 Determination of total saponin content

Total saponin content was estimated by extracting the sample (5 g) in alcohol and alcohol soluble part was concentrated to thick paste in rotary evaporator. This thick paste was sequentially extracted with petroleum ether (40-60 ^{0}C), chloroform, carbon tetrachloride and ethyl acetae each with 25 ml by reflux. All the solvent portions were discarded and residue was dissolved in 10 ml methanol.

Then acetone (50 ml) was slowly added to the above methanol soluble by gentle swirling the flask till the formation of precipitate completed. The content were allowed to settle for 10 min. and filtered and dried. Total content of the saponins was expressed in percentage.

2.6 DPPH free-radical scavenging activity

DPPH (1, 1- diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured by the method of Lamaison *et al.* [7]. The reaction mixture contained 1.5×10^{-7} M methanolic solution of DPPH and various concentrations of the test substances and were kept in dark for 50 min. Optical Density (OD) of the samples was measured at 517 nm against blank and IC_{50} values were calculated using linear regression analysis.

5-Lipoxygenase inhibitory activity (UV –Kinetic method):

In vitro 5-Lipoxygenase inhibition**:** 5-LOX enzyme inhibitory activity of *Xylia xylocarpa* extracts was measured using the method of Reddenna *et al.* [8] modified by Ulusu *et al* [9]. The assay mixture contained 80 mM linoleic acid and 10 µl potato 5-LOX in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to linoleic acid and the enzyme activity was monitored as the increase in absorbance at 234 nm. The reaction was monitored for 120 sec and the inhibitory potential of the test substances was measured by incubating various concentrations of test substances for two minutes before addition of linoleic acid. All assays were performed in triplicate. IC_{50} values were calculated using linear regression analysis.

3. Results and Discussion

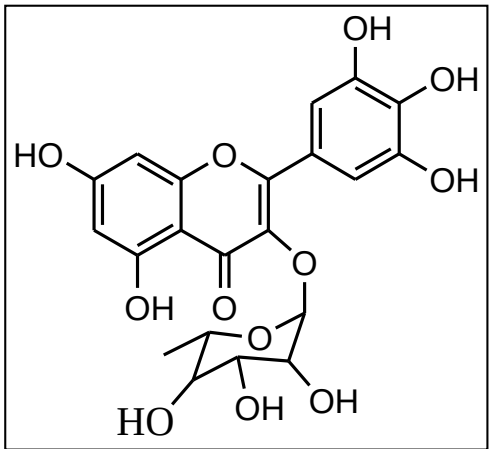
3.1 Polyphenol, flavonoid and saponin contents in leaf extract and its fractions of *Xylia xylocarpa:*

All the three phytochemical content were higher percentages in 90%methanol extract of *Xylia xylocarpa* leaves. Total Polyphenol content was about 44.2% which is reasonably good amount in plant material (Table 1). Total saponin content was above 50 %. Mimosaceae plants have been well characterized by impressive polyphenols and flavanoids diversity [10].

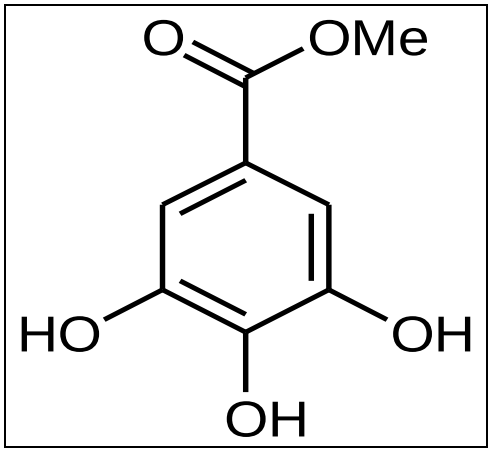
Table 1: Antioxidant and radical scavenging activity of leaf extract, its fractions and compounds of *Xylia xylocarpa*:

Type of extract	Total polyphenols %	Total flavonoids %	Total saponins %
90% methanol extract	44.20	4.17	54.5

Both leaf extract and its ethyl acetate fraction showed good scavenging activity. The IC_{50} values of sub fractions of ethyl acetate soluble part (Table 2) were equally comparable to standard – vitamin C IC_{50} (3.70). Two major compounds methyl gallate and myricitrin (Fig 1)were isolated from ethyl acetate soluble part column fractions and confirmed chemically and structurally (Mass, NMR, IR, TLC, HPLC)) by reported data [11, 12] and authentic standards (Fig 2). These two compounds also showed good antioxidant activity.



Myricitrin



Methylgallate

Fig 1: Isolated column fractions from ethyl acetate soluble part of *Xylia xylocarpa*

#	**Myrictirin[1] LI12802A $^{1}HD_6$-dmso**	**Myrictirin[1] LI12802A ^{13}C CD_3OD**	**Methylgallate *LI12801a ^{1}H CD_3OD***	**Methylgallate LI12801A ^{13}C**
1				121.59
2		156.41(C)	*6.943, s, 2H*	110.16
3		134.33(C)		146.53
4		177.78 (C)		139.78
5		161.32 (C)		146.53
6	6.211dJ=2Hz,1H	98.64(CH)		110.16
7		16414(C)		169.06
8	6.376dJ=2Hz,1H	93.48(CH)	*3.713, s, 3H*	52.25
9		157.46 (C)	*3.713, s, 3H*	52.25
10		104.06 (C)		
1'		119.67 (C)		
2'	6.896,s,1H	107.96(CH)		
3'		145.77 (C)		
4'		136.45 (C)		
5'		145.77 (C)		
6'	6.896,s,1H	101.96(CH)		
1"	5.209,s,1H	70.01 (CH)		
2"	3.991,m,1H	70.45 (CH)		
3"	3.562,m,1H	70.51 (CH)		
4"	3.165,ddd,1H	71.34 (CH)		
5"	3.388,m,1H	70.51 (CH)		
6"	0.85,dJ=6Hz,3H	17.48 (CH_3)		
5OH	12.692,s,1H			
7OH	10.60,s,1H			
3'5'OH	9.288,s,2H			
4'OH	8.863,s,1H			
2"OH	4.915,dJ=4.4Hz			
3"OH	4.681,dJ=4.8Hz			
4"OH	4.555,dJ=6.0Hz			
	465[M+H]$^+$		184	
IR	3747,3362,2356,1652,1612,1512,1200cm^{-1}		3463, 3326, 1694, 1618, 1540, 1469, 1314, 1196, 1037, 1003, 745, 756	
UV	257,354 nm		----	
MP	190-192°C			

Fig 2: Spectral data of the compounds Myrictirin & Methylgallate

The preliminary study on antioxidant activity and polyphenol content of selected species of Zingiberaceae [13] and Mimossaceae [14] reported that leaves of *Etiligera* species and leaves and stem bark of *Xylia xylocarpa* showed high antioxidant activity. It is also reported that this higher antioxidant activity was due to higher percentage of polyphenols and flavonoids percentages. Anti radical power of phenolic compounds are well known [15]. Phenolic compounds were reported to be very strong antioxidants. Antioxidant activity of phenolic compounds and plant extracts was closer to that of the synthetic antioxidant BHT. The organic and aqueous extracts leaf of *Artemisia herba alba* exerts strong antioxidant radical scavenging activity which were related to their polyphenol content [16]. The ethyl acetate extract of this plant contains good amount of flavonoids and phenolic acids that showed anti lipoperoxidant activity, antioxidant and free radical scavenging capacity. The present experimental data of *Xylia xylocarpa* also supported the same.

Table 2: Scavenging activity of DPPH radicals by extract, its fractions and compounds of *Xylia xylocarpa* leaf.

S. No.	Extraction / Fraction	DPPH : IC_{50} ug/ml
1	90% Methanol extract of leaf	7.8
2	Ethaylacetate soluble of 90%MeOH extract	5.9
3	Ethylacetate soluble part column fractions	
	i) Fraction-1(10% acetone in chloroform)	6.07
	ii) Fraction-2(30% acetone in chloroform)	15.0
	iii) Fraction-3 (50% acetone in chloroform)	3.7
	iv) Fraction-4 (acetone)	6.8
4	Column sub-fractions of fraction-2	
	i) Fraction-A	4.3
	ii) Fraction-B	5.7
	iii) Fraction-C	8.1
	iv) Fraction-D	9.0
5	Ethyl acetate insoluble part (aqueous phase)	18.2
6	Isolated compound-1(methylgallate)	2.8
7	Isolated compound-2(myricitrin)	4.3
8	Control –Standard – Vitamin-C	3.5

3.2 5-Lipoxygenase inhibitory activity

Ethyl acetate soluble part of *Xylia xylocarpa* leaf showing better inhibitory activity compared to crude extract. But column fractions of ethyl acetate soluble part didn't show that much inhibition of 5-Lixpoxygenase compared ethyl acetate soluble part (Table 3). Similarly isolated compounds methyl gallate and quercetin glycoside also gave IC_{50} of 5-lipoxygenase equivalent to ethyl acetate fraction.

Table 3: 5-Lipoxygenase inhibitory activity of extract, fractions and compounds of *Xylia xylocarpa* leaf.

S. No.	Extraction / Fraction	5-Lipoxygenase inhibition IC_{50} ugm/ml
1	90% Methanol extract of leaf	13.4
2	Ethaylacetate soluble of 90%MeOH extract	9.7
3	Ethylacetate soluble part column fractions	
	i) Fraction-1	15
	ii) Fraction-2	8.4
	iii) Fraction-3	8.3
	iv) Fraction-4	11.0
4	Column subfractions of fraction-2	
	i) Fraction-A	11.7
	ii) Fraction-B	13.4
	iii)Fraction-C	14.7
	iv)Fraction-D	14.2
5	Ethylacetate insoluble part (aqueous phase)	20
6	Isolated compound-1(methylgallate)	15
7	Isolated compound-2(myricitrin)	13.3
8	Control –De-methylated curcumin	3.7

The antiinflammatory activity of eithteen plant methanolic extracts was explained [17-20] by the potent inhibitory effects of their phenolic compounds on arachodonic acid metabolism through lipoxygenase pathway. Among the eighteen plants studied, *Bidens pilosa* (IC_{50} 21.8ug/ml) showed maximum, while *Emex australis* (IC_{50} 81.4) showed minimum inhibition of 5-Lipoxygenase inhibitory activity and the result revealed that there is direct correlation between phenolic content and antioxidant acivity. Antioxidants are known to inhibit plant lipoxygenase [21]. Studies have indicated the oxygen free radicals in the process of inflammation and phenolic compounds may block the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activity and may serve as scavenger of reactive free radicals which are produced during arachindonic acid metabolism [22-24]. The inhibitory activities of this plant extracts and its ethyl acetate fraction may also be due to the compounds i.e., myricetin and methylgallate isolated from those extracts. It is noticed that methyl gallate has dual cyclooxygense-2 and 5-lipoxygenase inhibitory activity which might provide the basis for novel antiinflammatory drugs [25].

4. Conclusion

It is concluded from the above research work that 90% methanol extracts and subsequent fractions and isolated compounds of leaves of *Xylia xylocarpa* showed potent inhibitory activities on tyrosinase and 5-Lipoxygenase and potent antioxidant activity. Also our earlier work on antimicriobial study (unpublished) of the same plant leaf extracts showed good antibacterial activity.

5. Reference

1. Duflour D, Pichette A, Mshvildadze V, Brodette H, Ubert ME, Lavoic S *et al*. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Ledium groenlandicum* Retizius. J Ethanopharmacol. 2007; 111:22-28.
2. Salfarina R, Supawan B, Tansaringkarn N, Ruangrungsi. Screening for free radical scavenging activity from ethanol extracts of Mimosaceous plants endemic to Thailand. J Health Res. 2008; 22(2):55-59.
3. Asanuma M, Miyazaki I, Ogawa N. Dopamine or L-Dopa induced neurotoxicity: The role of dopamine quinine formation and tyrosinase in model of pakinson disease. Neurotox Res. 2003; 5:165-176.
4. Jager AK, Hutchings A, Van Staden J. Screening of Zulu medicinal plants for protstaglandin synthesis inhibitiors. J Ethanopharmacol.1996; 52:95-100.
5. Bouriche H, Miles EA, Selloum L, Calder PC. Effect of cleome Arabica leaf extract rutin and quercetin on soyabean lipoxygenase activity and on generation of inflammatory eicosanoids by uman neutrophils. Prostagladins Leukot essential fatty acids. 2005; 72:195-201.
6. Gramann J, Hippeli S, Dornisch K, Rohnert U, Beuscher N, Elstner EF. Antioxidant properties of essential oils. Arzneim. Forsch.//Drug. Res. 2000; 50(1):135-9.
7. Sidduraju P, Vijayakumari K, Janardhan K. Nutrient and chemical evaluation of raw seeds of *Xylia xylocarpa*: An underutilized food source. Food Chem. 1995; 53:299-304.

8. Yoshimura M, Watanabe Y, Kasai K, Yamakoshi J, Koga T. Inhibitory effect of an ellagic acid rich pomegranate extract on tyrosinase activity and ultraviolet-induces limentation. Biosci Biotechnol Biochem. 2005; 69:2368-2373.
9. Reddenna P, Whelan J, Maddipati KR, Reddy CC. Purification of arachinonate 5-Lipoxygenase from potato tubers. Methods Enzymol. 1990; 187:268-277.
10. Ulusu NN, Ercil D, Sakar MK, Tazean EF. Abietic acid inhibits lipoxygenase activity. Phytother Res. 2002; 16:88-90.
11. Evans WC. Pharmacognosy. Harcourt pubilications Ltd. United Kingdom. 2002, 289-393.
12. Krishnaraju AV, Rao CV, Sundararaju D, Subbaraju GV. Antioxidant Constituents from Stem Galls of Quercus Infectoria Olivier. Asian J Chem. 2005; 17(3):1883-1838.
13. Shin-Kyo Chung, Young-Chan Kim, Yoshiaki Takaya, Kenifji Terashima, Masataka Niwa. Novel flavonol glycoside 7-O-methyl mearnsitrin from *Sageretia theezans* and its antioxidant effect. J Agric food chem. 2004; 52:4664-4668.
14. Chan EWC, Lim YY, Wong LF, Lixnto FS, Wong SK, Lim KK *et al*. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. Food Chem. 2008; 109:477-483.
15. Salfarina R, Supawan B, Tanasorn T, Nijsiri R. Screening for free radical scanvenging activity from ethanolic extract of Mimosaceous plants endemic to Thailand. J Helath Res. 2008; 22(2):55-59.
16. Pietta PG. Flavanoids as antioxidant. J Nat Products. 2000; 63:1035-1042.
17. Khennouf Seddik, Irantini Nadget, Baghiani Abderrahmane. Antioxidant and antibacterial activities of extracts from Artemisia herba alba ass. Leaves and some phenolic compounds. J Med Planat Res. 2010; 4(13):1273-1280.
18. Sanchez-Ferrer A, Rodringuez-Lepez JN, Garcia-Canovas F, Garcia-Carmina F. Tyrosinase: a comprehensive review of its mechanisms. Biochim Biophys Aacta.1995; 1247:1-11.
19. KimYC, Kim MV, Takaya Y, Niwa M, Chung SK. Phenolic antioxidant isolated from mulberry leaves. Food Sci Biotech. 2007.
20. Kubo I, Kinbt-Hori I. Flavanols from saffron flower: Tyrosinase inhibitory and inhibition mechanism. J Agric Food Chem.1999; 47:4721-4125.
21. Umashankar A, Bharti O. In vitro 5-Lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. Journal of Medicinal Plants Research. 2008; 2(9):207-212.
22. Ammon PT, Safayhi H, Mack T, Sabieraj J. Mechanism of anti-inflammatory actions of curcumine and boswellic acids. J Ethanopharmacology. 1993; 38:113-119.
23. Trouillas P, Calliste CA, Allais DP, Simon A, Marfack A, Delage C *et al*. Antioxidant, antiinflammatory and antiproliferative properties of sixteen water plants extracts used in the Linousin countryside as herbal teas. Food Chem. 2003; 80:399-407.
24. Sreejayan N, Rao MNA. Free radical scavenging activity of curcuminoids. Arzneimittalfors Chung. 1996; 46(2):169-171.
25. Sejong kim, Meihuajin, Eunkyung Lee, Tae Chua Moon, Zhejiu Quan, Juhyeyang, Kun Hoson, Kil-UngKim, JongKson, Hyeun Wook Chang. Effects of methyl gallateon arachidonic acid metabolizing enzymes: Cyclooxygenases – 2 and 5-Lipoxygenase in mouse bone marrow derives mast cells. Arch Pharm Res. 2006; 29(10):874-878