IJPSR (2020), Volume 11, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 April 2019; received in revised form, 19 July 2019; accepted, 25 December 2019; published 01 January 2020

PHYTOCHEMICAL STUDIES, FTIR AND GC-MS ANALYSIS OF *HARDWICKIA BINATA* ROXB. (FABACEAE / CAESALPINIACEAE)

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Keywords:

Hardwickia binata, Phytochemicals, FTIR, GC-MS analysis

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ABSTRACT: *Hardwickia binata* belongs to family Fabaceae / Caesalpiniaceae and commonly known as 'Anjan'. The present study includes phytochemical screening, FTIR and GC-MS analysis. Shade dried powdered leaves, seed and husk of *H. binata* extracted in petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, distilled water using Soxhlet apparatus and used for phytochemical analysis. Crude powder of plant used for FTIR analysis and ethanolic extracts was analyzed using GC-MS. The phytochemical screening of *H. binata* revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, lipids, tannins, phenolic compounds, quinones, mucilage, volatile oils, fats, and fixed oils whereas alkaloids, emodins and resins were not detected in any extracts of leaves, seed, and husk. The majorities of compounds are present in acetone, methanol, ethanol and distilled water and glycosides, lipids and mucilage are present in all extracts. FTIR analysis showed the presence of alcohol, phenols, amines, amides, carboxylic acids, aromatics, alkenes, alkanes, aliphatic amines, esters, ethers, alkynes, alkyl halides in leaves, seed and husk; saturated aliphatic found in only seed and aldehydes only in husk and GC-MS analysis confirmed ten different chemical compounds 1,1-diethoxy-ethane, tetradecamethyl-cycloheptasiloxane, linoleic acid, ethyl ester, ethyl oleate in leaves, seed and husk; hexadecanoic acid and ethyl ester found in only leaves and seed; 1-methyl-4 -(1-methylethyl)-Benzene, 2-methyl-5-(1-methylethyl)-Phenol, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-o1 are found in only leaves; dodecamethyl-cyclohexasiloxane only in seed; thymol only in husk shows various biological activities. The different extracts of *H. binata* revealed several phytochemical components as a rich source of pharmaceutically and biologically important bioactive compounds that may build up new medicines with more efficiency.

INTRODUCTION: The plant kingdom is a significant part of potential drug an inexhaustible source of bioactive constituents. Preliminary phytochemical screening is a valuable step in the detection of the bioactive compounds present in plants.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(1).233-40

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(1).233-40

Phytochemicals are known to provide protection against insect attacks and plant diseases. *Hardwickia binata* is a deciduous, moderate to large-sized tree. The bark of saplings almost silvery-white and smooth. Leaves are alternate, bifoliolate resembling Bauhinias. Leaflets are almost kidney-shaped and grayish-green in color, sessile, entire, obliquely ovate and coriaceous.

Flowers are small, yellowish-green in an axillary and terminal lax panicled raceme. The fruit is a flat, samaroid pod containing one seed near the apex. Seed is flat; straight to slightly bended pointed at one end and rounded at the other, with a fairly hard

testa [1, 2]. Previous researchers worked out tannins from the bark are used to produce medicines for the treatment of diarrhea, worms, indigestion and leprosy and also produce an appetizer [3]. The leaves, pods and bark contain tannins [4].

Bark used for gums and resins [5]. The leaves are used for fodder and manure [6]. The leaves extract showed activity against both gram-positive and gram-negative bacteria and fungi. Bioactive substances showed antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, pneumonia, eye infections and mycotic infections and tannins have been traditionally used for protection of catarrh, wounds, hemorrhoids, diarrhea and antidote in heavy metal poisoning. Flavonoids possess anti-inflammatory, anti-allergic, antithrombotic, anti-microbial and vasoprotective effects [7]. The leaves are used for headache and treatment of constipation [1, 7]. The leaves had crude protein [8, 9]. The bark and leaf used as a source of medicine for rheumatism [10]. In *H. binata* an investigation was made of the antioxidants and their protective mechanism in chloroplasts against SO_2 pollution [11]. The phenolic compounds' role in giving protection to the plants against deleterious effects of UV rays and certain phytopathogenic microorganisms. The protein, phenols and flavonoids can be used as chemical markers in taxonomic studies [12]. Phytochemical analysis studies the presence of active compounds. Hence, the present investigation was undertaken to work out preliminary phytochemical studies, FTIR and GC-MS analysis with the aim to report the functional components of leaves, seed and husk of *Hardwickia binata* Roxb.

MATERIALS AND METHODS:

Collection and Identification of Plant Material: The plant materials such as leaves and pods of *Hardwickia binata* were collected from different localities *viz.* Sangali (Tal-Atpadi, Swatantrapur vasahat), Dhule (tal-dhule, borvihir Area) and Jalgaon (Tal-Muktainagar, Muktainagar forest) Districts in April 2015. Taxonomic identification of the plant was done and herbarium was deposited to the Department of Botany, Shivaji University, Kolhapur.

Preparation of Extracts: The collected materials (leaves and pods) of *Hardwickia binata* were shade dried and coarsely powdered in a mixer grinder, sieved and used for extraction. The leaves, seed and husk powder were extracted separately with petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, and distilled water in the increasing order of their polarity by using Soxhlet apparatus. The extract was decanted, filtered through Whatman No.1 filter paper, concentrated and preserved in airtight bottles until further use.

Phytochemical Studies: The qualitative phytochemical tests for the different solvent extracts were used for the identification of active chemical constituents like carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, lipids, tannins, phenolic compounds, quinones, mucilage, volatile oils, fats and fixed oils. Preliminary phytochemical analysis tests for presence or absence of various phytoconstituents.

FTIR Analysis: The Fourier Transform Infrared (FTIR) spectroscopy analysis was performed to know the occurrence of chemical bonds or functional groups present in the crude powder of leaves, seed, and husk of *Hardwickia binata.* The peak values of FTIR were recorded and their corresponding functional groups were identified.

GC-MS Analysis: The chemical composition of ethanolic extracts of leaves, seed, and husk of *Hardwickia binata* was determined by gas chromatography-mass spectrometry (GC-MS) as it provides enhanced sample identification, higher sensitivity, an increased range of analyzable samples and faster results. This helps to constitute the chemical picture of plant extracts and by which the complex mixtures can be resolved into individual components.

RESULTS AND DISCUSSION:

Phytochemical Studies: The preliminary phytochemical screening of *H. binata* revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, lipids, tannins, phenolic compounds, quinones, mucilage, volatile oils, fats and fixed oils whereas alkaloids, emodins and resins were not detected in any extracts of leaves, seed and husk **Table 1**. Previous results revealed that the presence of saponins, tannins, steroids, terpenoids, phenolic groups, coumarin, flavonoids and carbohydrates.

Alkaloids were found to be absent in petroleum ether, chloroform, and ethanolic leaves extracts of *H. binata* [1]. A similar type of analysis has been documented in ethanolic leaves extracts of *H. binata* but alkaloids and anthraquinones were not present [7]. The phytochemical study of leaves and seeds of *Bauhinia racemosa*, *Bauhinia purpurea* and *Hardwickia binata* in ethanolic extracts are the presence of phenols, saponins, flavonoids, glycosides and tannins while alkaloids were not detected [12].

The phytochemical screening of leaves extract of *Bauhinia variegata* with petroleum ether, chloroform, ethanol, and water showed the presence of carbohydrates, gums, proteins, amino acids, fats and oils, triterpenoids, steroids, glycosides, flavonoids, alkaloids, tannins [13]. Similar type of analysis has been documented in *Bauhinia racemosa* leaves [14, 15].

The various extracts of root bark exudates of *H. binata* show the presence of carbohydrates, glycosides, fixed oils and fats, proteins and amino acids, saponins, tannins, phytosterols, alkaloids, phenolic compounds, flavonoids while gums and mucilage are absent [16].

In present work confirmed the previous reports. The majorities of compounds are present in acetone, methanol, ethanol, and distilled water and glycosides, lipids and mucilage are present in all extracts.

TABLE 1: QUALITATIVE PHYTOCHEMICAL INVESTIGATION OF *H. BINATA*

Chemical tests	Solvent Type						
	P. E.	Chlo.	E. A.	Acetone	Methanol	Ethanol	D. W.
	L S H	L S H	L S H	L S H	L S H	L S H	L S H
Carbohydrates							
Benedict's	- - -	- - -	- - -	- - -	- - -	- - -	+ + +
Fehling's	- - -	+ - -	- - -	- - -	- - -	+ + -	+ + +
Proteins							
Xantho-P.	- - -	- - -	+ + +	+ + +	- + +	+ - +	+ + +
Millon's	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
Amino acids							
Ninhydrin	- - -	- - -	- - -	- + +	- + +	- + +	+ + +
Cystein	- - -	- - -	- - -	- - -	- + +	- + +	- - -
Steroids							
Salkowski	- - -	- - -	- - -	- - -	+ + +	+ + +	+ + +
Glycosides							
Kellar-killani	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Foam T	- - -	- - -	- - -	- - -	- + +	+ + +	+ + +
Flavonoids							
Shinoda	- - -	- - -	- - -	- + +	+ + +	+ + +	+ + +
$FeCl_3$	- - -	- - -	+ - +	+ + +	+ + +	+ + +	+ + +
Leadacetate	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
Alkaloids							
Mayer's	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Wagner's	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Tannins and phenolic com.							
Dilute I_2	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
Dilute HNO_3	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
Quinones							
Con. HCL	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
Emodins							
NH_4OH	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Mucilage							
Cold H_2O	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Volatile oils							
Alcohol	- - -	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Fats and Fixed oils							
Chloroform	+ + +	+ + +	+ + +	+ + +	- - -	- - -	- - -
Resins							
Acetone	- - -	- - -	- - -	- - -	- - -	- - -	- - -

(+) Positive test, (-) negative test; L- leaves, S- seed, H- husk; P. E- petroleum ether, chlo-chloroform, E. A- ethyl acetate, D.W-distilled water

FTIR Analysis: The results revealed in the crude powder of leaves, seed and husk of *H. binata* gave the characteristic absorption peaks as represented in **Fig. 1** and **Table 2**. The crude powder of *H. binata* FTIR confirmed the presence of alcohol, phenols, amines, amides, carboxylic acids, aromatics, alkenes, alkanes, aliphatic amines, esters, ethers, alkynes, alkyl halides in leaves, seed and husk; saturated aliphatic found in only seed and aldehydes only in husk. FTIR spectra of untreated and alkali-treated *Hardwickia binata* fibers were determined the chemical composition of cellulose, hemicellulose and lignin [17]. The methanolic extracts of *Myristica dyctyloides* fruit have FTIR spectra showed the presence of functional group in all the extracts which have medicinal properties [18].

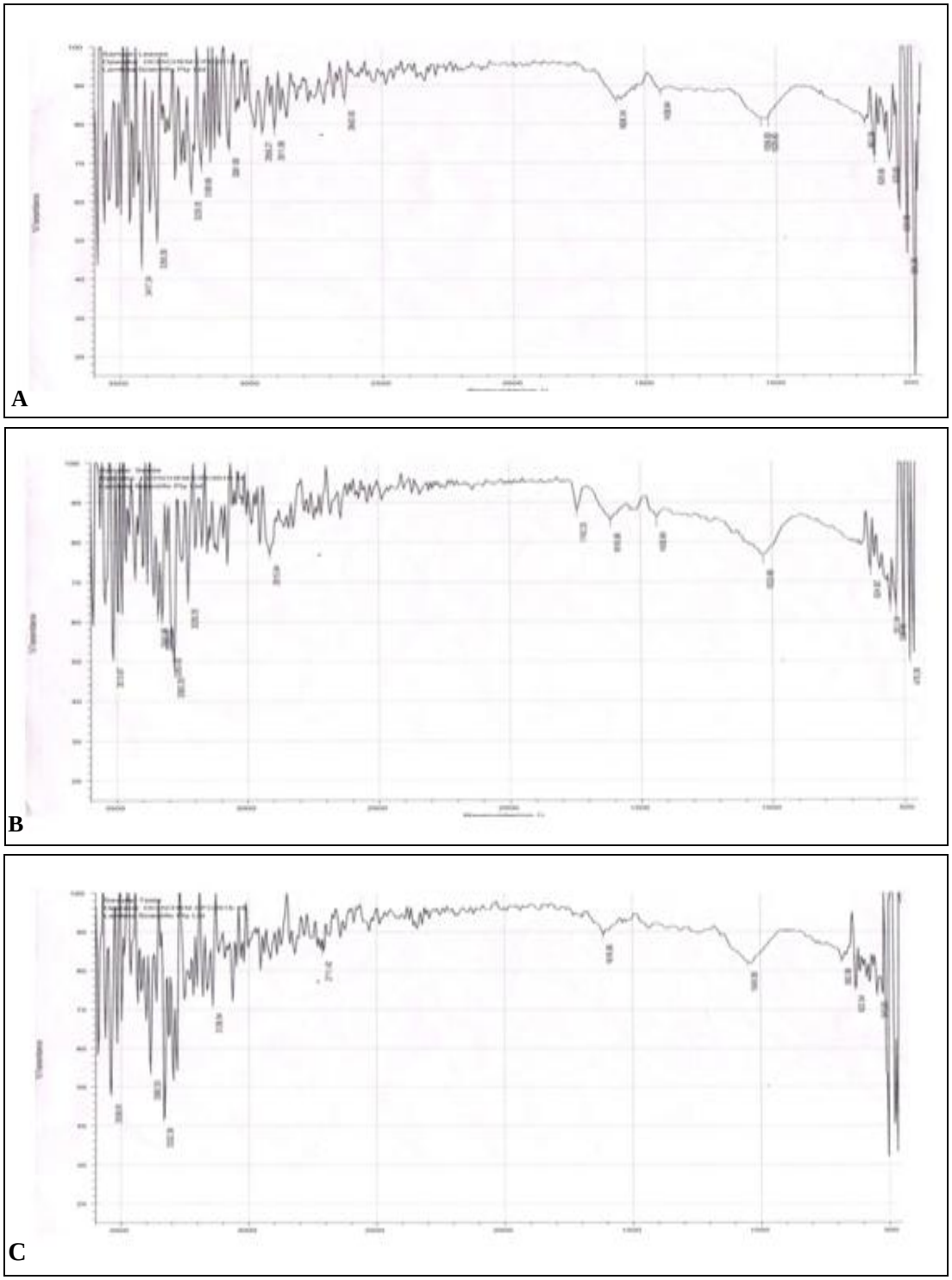

FIG. 1: FTIR SPECTRUM ANALYSIS OF CRUDE POWDER OF HARDWICKIA BINATA (A) LEAVES, (B) SEED, (C) HUSK

TABLE 2: FTIR ANALYSIS OF CRUDE POWDER OF *H. BINATA*

S. no.	Leaves P. V.	Functional group	Seeds P. V.	Functional group
1	3417.24	Alcohols, Phenols	3513.67	Alcohols
2	3359.39	Alcohols, Phenols; Amines, Amides	3343.96	Amines, Amides; Alcohols, Phenols
3	3228.25	Amines, Amides; Carboxylic acids	3328.53	Alkynes, Amines, Amides
4	3189.68	Carboxylic acids	3293.82	Alkynes, Amines, Amides
5	3081.69	Aromatics; Alkenes; Carboxylic acids	3282.25	Alkynes, Amines, Amides
6	2958.27	Alkanes	3228.25	Alcohols, Phenols
7	2911.99	Alkanes	2915.84	Alkanes
8	2642.00	Carboxylic acids	1743.33	Esters, saturated aliphatic
9	1608.34	Amines	1616.06	Amines
10	1438.64	Aromatics	1438.64	Aromatics
11	1056.80	Aliphatic amines; Alcohols, Carboxylic acids, Esters, Ethers	1033.66	Aliphatic amines; Alcohols, Carboxylic acids, Esters, Ethers
12	1029.80		624.82	Alkynes; Alkyl halides
13	663.39	Alkynes; Alkyl halides	551.54	Alkyl halides
14	628.68	Alkynes; Alkyl halides	528.40	Alkyl halides
15	570.83	Alkyl halides	478.26	Alkyl halides
16	532.26	Alkyl halides		
17	505.26	Alkyl halides		
	Husk P. V.			
18	3536.81	Alcohols	1616.06	Amines
19 & 20	3382.53 3332.39	Amines, Amides; Alcohols, Phenols	1049.09 682.68	Alcohols, Carboxylic acids, Esters, Ethers; Al.amines Alkynes; Alkyl halides
21	3139.54	Carboxylic acids	632.54	Alkynes; Alkyl halides
22	2711.42	Aldehydes	543.83	Alkyl halides

FTIR- Fourier transform infrared spectroscopy; P.V. - peak values

GC-MS Analysis: GC-MS analysis confirmed the results as shown in **Table 3** and **Fig. 2**. It showed the presence of 10 major groups. 1, 1-diethoxy-ethane, tetradecamethyl- cycloheptasiloxane, linoleic acid, ethyl ester, ethyl oleate in leaves, seed and husk; hexadecanoic acid and ethyl ester found in only leaves and seed; 1-methyl-4-(1-methylethyl)-Benzene, 2-methyl-5-(1-methylethyl)-Phenol, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-o1 are found in only leaves; dodecamethyl-cyclohexasiloxane only in seed; thymol only in husk is the pharmaceutically important. The different biochemical compounds identified by GC-MS show various biological activities shown in **Table 4**.

TABLE 3: GC-MS ANALYSIS OF ETHANOLIC EXTRACTS OF LEAVES, SEED AND HUSK OF *H. BINATA*

S. no.	Name of compounds	MF	MW	Part used					
				Leaves		Seed		Husk	
				R. T.	Area%	R. T	Area%	R. T	Area%
1	1,1- diethoxy-Ethane	$C_6H_{14}O_2$	118	6.336	11.68	6.295	39.52	6.333	19.81
2	1-methyl-4-(1methylethyl)-benzene	C10H14	134	11.747	7.46	-	-	-	-
3	2-methyl-5-(1-methylethyl)-phenol	$C_{10}H_{14}O$	150	16.020	28.15	-	-	-	-
4	Thymol	$C_{10}H_{14}O$	150	-	-	-	-	16.041	58.56
5	Dodecamethyl-cyclohexa siloxane	$C_{12}H_{36}O_6Si_6$	444	-	-	16.159	15.77	-	-
6	Tetradecamethyl cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	518	18.489	5.18	18.494	4.23	18.492	11.13
7	3, 7, 11, 15-Tetramethyl-2hexadecen-1-o1	$C_{20}H_{40}O$	296	23.594	10.37	-	-	-	-
8	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	26.655	7.04	26.658	8.62	-	-
9	Linoleic acid, ethyl ester	$C_{20}H_{36}$	308	30.213	20.61	30.218	20.35	30.213	6.98
10	Ethyl oleate	$O_2C_{20}H_{38}O_2$	310	30.298	9.50	30.303	11.51	30.293	3.52

GC-MS- Gas Chromatography - Mass Spectrometry, R.T. - Retention time, MF- Molecular Formula, MW- Molecular Weight

The GC-MS analysis of leaves of *H. binata* was carried out on the petroleum ether, chloroform and ethanolic extracts and among all the extracts, ethanolic extracts showed the presence of maximum chemical constituents [1]. The GC-MS analysis of methanolic extracts in root bark exudates of *H. binata* identified 22 compounds [16].

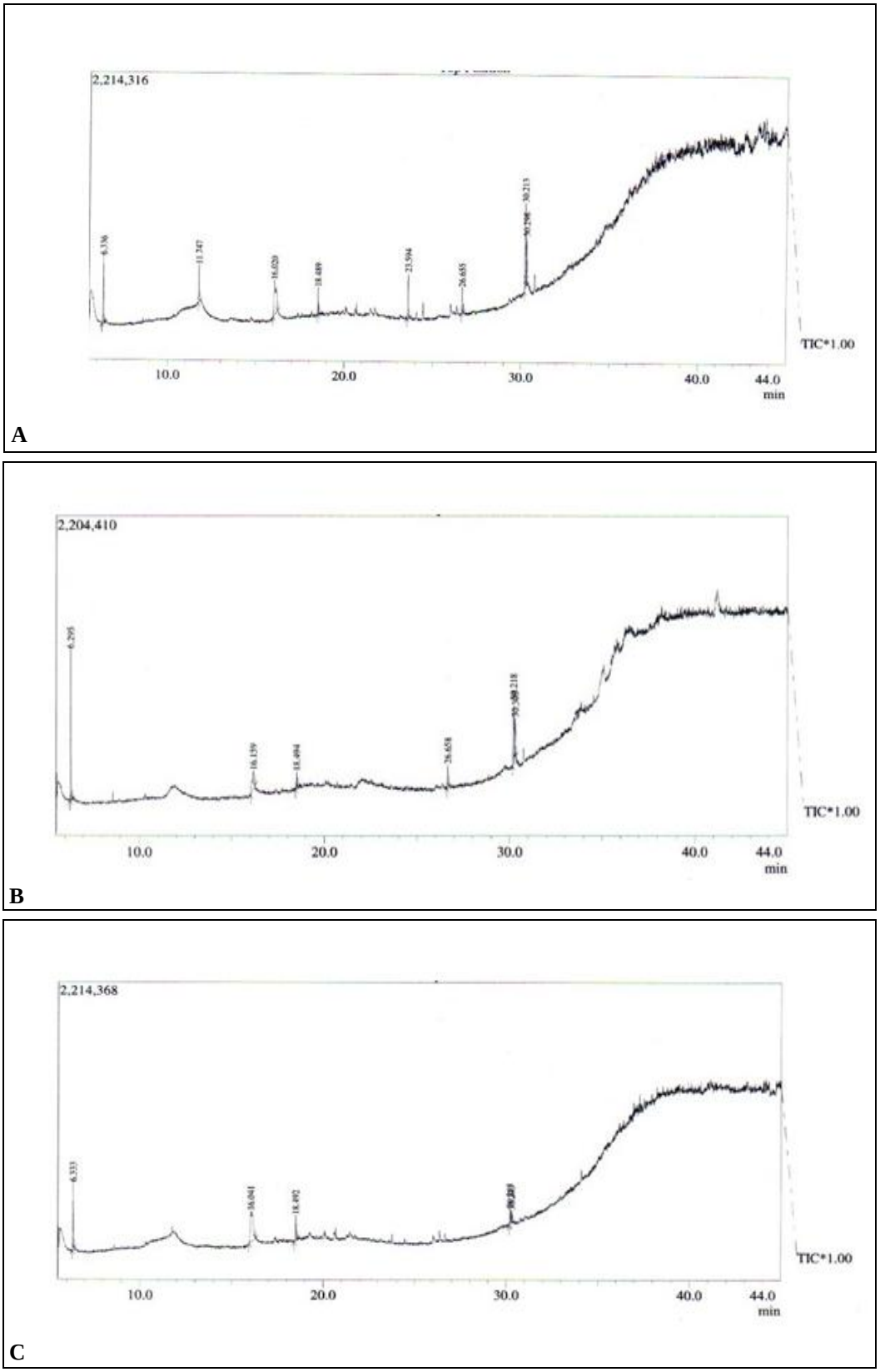

FIG. 2: GC-MS CHROMATOGRAM OF ETHANOLIC EXTRACT OF *HARDWICKIA BINATA* (A) LEAVES, (B) SEED, (C) HUSK

TABLE 4: ACTIVITY OF BIOACTIVE COMPOUNDS IDENTIFIED BY GC-MS STUDY OF LEAVES, SEED AND HUSK OF *H. BINATA*

Name of compounds	Activity
1, 1-diethoxy-Ethane	Flavoring agent [19]
Thymol	Antioxidant, free radical scavenging, anti-inflammatory, analgesic, antispasmodic, antibacterial, antifungal, antiseptic and antitumor [20]
Dodecamethyl-Cyclohexasiloxane	Antioxidant, antifungal, antibacterial, anti-inflammatory, antimicrobial [21, 22]
TetradecamethylCycloheptasiloxane	Antimicrobial [22]
3,7,11,15-Tetramethyl-2-hexadecen-1-o1	Cancer preventive, anti-inflammatory, fragrance compound, antimicrobial [23]
Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor [24]
Linoleic acid, ethyl ester	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge [25]
Ethyl Oleate	Cancer preventive, flavor, hypocholesterolemic, 5-Alpha reductase inhibitor, antiandrogenic, perfumery, insectifuge, anti-inflammatory, anemiagenic, dermatitigenic, choleretic [26]

CONCLUSION: The present investigation of *H. binata* revealed the presence of many phytochemical components as a rich source of pharmaceutically and biologically important bioactive compounds that may build up new medicines. FTIR spectra showed the various functional groups may serve as a new potential source of medicines with more efficiency. 10 compounds that have been screened by a GC-MS it will help to therapeutic activity. Various studies confirmed that phytochemicals contribute medicinal properties that are of great importance as a good source of new useful drugs. The quantitative analysis of these phytocomponents will be an interesting area for further study.

ACKNOWLEDGEMENT: Authors are thankful to the Principal, Yashavantrao Chavan Institute of Science, Satara for providing the facilities for research work. The authors also thank the Head, Department of Botany for their support and cooperation during the research work.

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Deshmukh SV and Ghanawat NA: Phytochemical studies, FTIR and GC-MS analysis of *Hardwickia binata* roxb (fabaceae/ caesalpiniaceae). Int J Pharm Sci & Res 2020; 11(1): 233-40. doi: 10.13040/IJPSR.0975-8232.11(1).233-40.