Original Article

GC-MS Analysis and *In vitro* Cytotoxicity Studies of Root Bark Exudates of *Hardwickia binata* Roxb.

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ABSTRACT

Hardwickia binata Roxb. (Fabaceae) root bark exudate has been traditionally used by tribes of Chitteri hills to cure breast cancer. The main objective of this study is to give a scientific validation to the traditional use of *H. binata*. The root bark exudates of *H. binata* was collected from Chitteri hills, shade dried, coarsely powdered and extracted with methanol using soxhlet apparatus. The Physico chemical properties, qualitative phytochemical studies, quantitative phytochemical studies and GC-MS analysis was carried out. The cytotoxic effect of methanol extract of *H.binata* root bark exudate was studied against cancer cell lines by MTT assay. The phytochemical studies indicated the presence of flavonoids, saponins, phenols and tannins. GC-MS study also revealed the presence of 22 organic compounds out of which 18 compounds were reported with various activities and five compounds reported to possess anticancer activity. *In vitro* cytotoxic activity on the cell lines showed more degree of inhibition against African Green Monkey Kidney Epithelial cells (Vero), Human cervical cancer cell lines (HeLa) and Human breast cancer cells (MCF-7).

Keywords: Anticancer, Cytotoxicity, *Hardwickia binata,* Phytochemical, Cell lines.

INTRODUCTION

Cancer is a dreadful disease characterized by the irregular proliferation of the living cells[1]. Though chemotherapy is now being generally used as a standard treatment method, search for anticancer agents from natural products has also been increased over the years because plants could exaggerate to diminish the toxicity

caused due to chemotherapy[2-3]. The plants containing anticancer properties would benevolently play a vital role in the discovery of potential drugs for treating cancer[4]. Phytochemicals have always been sought after because of their inherent potential to cure disease, as demonstrated by ancient medicinal practices[5-7]. The present study aimed to provide a scientific validation to the traditional use of root bark exudates of *Hardwickia binata* Roxb. by Malayali tribes of Chitteri hills against cancer.

Hardwickia binata Roxb. is a native species of tropical south – Southern East Asia. The synonyms are *Hardwickia trapeziformis* R. Grah. and *Harongana madagascariensis* Choisy. It grows in dry and hot climate, characterised by a long period of drought, low to moderate rainfall. It is a deciduous, moderate to large-sized tree, extremely hard, heavy and durable timber. The wood is largely used for beams and mine props, bridge and house construction, agricultural implements, carts and wooden wheels and railway sleepers. Leaves are used as cattle fodder.

MATERIALS AND METHODS

Preparation of Extracts

The root bark exudates of *H. binata* Roxb. were collected from the Chitteri, Southern, Eastern Ghats, Tamil Nadu. Chitteri hills. The Chitteri hills are situated towards North East of Salem district within the geographical limit of 78°51'10" - 78°32'40" E, longitude and 11°55'14"-12 °4'48" N, latitude and occupy an area of about 654.22 Km^2. The average height of the Chitteri hills is 3600ft. The plant was identified with the help of local flora and they were authenticated by taxonomists. The voucher specimens were also submitted to Vivekanandha College Herbarium (Ref. No. Angio-514). The exudates were dried in shade and coarsely powdered. The plant powder was sieved through 40µm sieve plate and the fine powder was used for extraction. The root bark exudates were extracted with Soxhlet apparatus by a continuous hot percolation process with various solvents such as petroleum ether, chloroform, acetone, alcohol and water, separately. After completion of extraction, the extract was filtered and the solvent was removed by evaporating in a water bath. The extract was stored in desiccators for further study.

Phyto chemical studies of *Hardwickia binata* Roxb.

Physico-chemical studies, qualitative and quantitative phytochemical tests were carried out in the methanol extract of root bark exudates of *H. binata* using standard methods[8-12]

GC-MS analysis

The Gas chromatography–mass spectroscopy (GC–MS) analysis affords the advantage of identifying the chemical entities present, which constitutes the chemical picture of a plant (herbal) extract and by which the complex mixtures can be resolved into individual components. GC-MS analysis of the methanol extract of *H.binata* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS)[13] equipped with a Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in the electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110°C (isothermal for

2min.), with an increase of 10°C/min. to 200°C, then 5°C/min. to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min., and the total GC/MS running time was 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was the Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was the Turbo-Mass over-5.2.

Pharmacological studies on *Hardwickia binata*

Cell culture has been used to screen anticancer activity, since there is a clear correlation between *In vitro* and *In vivo* activities of potential chemotherapeutic agents. There is a scientific justification for cytotoxicity testing in tissue culture, since animal models are in many ways inadequate for predicting the effects of chemicals on humans since there are many metabolic differences between two. Cytotoxicity studies involve the analysis of morphological damage or inhibition of the zone of outgrowth induced by the chemicals tested.

In vitro cytotoxicity studies

The Antiproliferative activity of root extracts was tested by MTT [(3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazo-liumbromide] assay. MTT measures the metabolic activity of viable cells. The assay was nonradioactive and could be performed entirely in a micro titer plate (MTP). It is suitable for measuring cell proliferation, cell viability and cytotoxicity. This method was based on the principle that viable cells convert MTT into a formazan[14] an insoluble salt, which is solubilised and quantified. Increase in its concentration indicates the increased number of viable cells. The absorbance directly correlated with the viable cell number.

RESULT AND DISCUSSION

The physico-chemical evaluation of drugs (Table 1) is an important parameter in detecting adulteration or improper handling of drugs. The total ash value was 0.47g/dr.wt., acid insoluble ash was 0.27g/dr.wt., sulphated ash was 1.6g/dr.wt. and water soluble ash was 0.37g/dr.wt. The extractive values are studied with two solvents such as ethanol and water. The extractive value of ethanol and water was 0.65g and 0.67g respectively. The loss on drying crude drug of root barks exudates of *H. binata* was 0.94g/g fr. wt. Qualitative and quantitative analysis results are given in Table 2 & 3.

GC-MS studies

The GC-MS analysis led to the identification of 22 compounds from the gas chromatography fractions of the methanol extract of *H. binata.* The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the methanol extract are presented in Table 4 and Fig. 2.

The components detected in methanol extract were Methoxydi (1-pyrrolidinyl) phosphine, an alkaloid, which is found at retention time of 6.51and peak area was 0.02%; 1-tert-Butyl-3-(3-methoxyphenyl)-bicyclo [1.1.1], a pentan, that is found at retention time of 7.72 and peak area was 0.32%; Limonene dioxide is found at retention time of 10.88 and peak area was 1.10; 2-[3-Cyclohexylaminopropylamino] ethylthiophosphate is a sulphur compound found at retention time of 11.86 and peak area was 0.02%; and Pentanoic acid 2-(2-hydroxy-2-methyl-4-phenylbut-3- an amino compound is found at retention time of 12.10 and peak area was 2.03 %.

Decanoic acid ethyl ester is a fatty acid ester that is found at retention time of 12.70 and peak area was 17.55; Cis-9-Hexadecenal is an aldehyde compound which is found at retention time of 14.27 and peak area was 58.68%; 4-Octadecenal is an aldehyde compound that is found at retention time of 14.34 and peak area was 35.67%; N-[3-[6-Hydroxyhexyl]aminopropyl] aziridine is an amino compound which is found at retention time of 14.53 and peak area was 2.74%; 9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z) which is a linolenic acid ester that is found at retention time of 14.69 and peak area was 3.91%; 9,12-Octadecadienoyl chloride(Z,Z) which is a linoleic acid compound that is found at retention time of 14.95 and peak area was 36.47%; 4-Hexenoic acid 2-amino-6-hydroxy-4-methyl an amino compound that is found at retention time of 15.33 and peak area was 0.05%; 9,12,15-Octadecatrienoic acid ethyl ester, (Z,Z,Z) a linolenic acid ester that is found at retention time of 15.90 and peak area was 2.10%; 8,11,14-Eicosatrienoic acid, (Z,Z,Z) is an unsaturated fatty acid is found at retention time of 16.99 and peak area was2.61%; 5,8,11,14-Eicosatetraenoic acid ethyl ester (all-Z) an unsaturated fatty acid ester is found at retention time of 17.10 and peak area was 15.08%; 2H-Pyran-3-ol 2-ethoxy-3 4-dihydro- acetate an alcoholic compound is found at retention time of 17.12 and peak area was 0.11%.

3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino] propionitrile is a nitrogen compound found at retention time of 18.05 and peak area was 0.03%; Deoxyspergualin a nitrogen compound that is found at retention time of 19.44 and peak area was 0.06%; 1H-3a 7-Methanoazulene octahydro-1,4,9,9-tetramethyl a nitrogen compound is found at retention time of 19.66 and peak area was 1.38%; Benzoic acid 4-nitro-1-methylethyl ester is an aromatic acid compound found at retention time of 20.03 and peak area was 0.58%; Squalene a triterpene which is found at retention time of 23.76 and peak area of was 4.78%. The peak area ranged from 0.02 to 58.68 percentages for reporting compounds.

The therapeutic activity of various compounds was reported based on Dr.Dukes's phytochemical and ethnobotanical database. Out of the 22 compounds identified from the extract, 18 compounds were reported to be active. Most of the compounds have been reported to have antimicrobial activity. Many of them have been shown to be Hepato protective, Cardio protective while some have anti-inflammatory property also. Among the various bioactive compounds present, the following five have been identified and reported to have anticancer properties. They are 9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z)-), 9,12,15-Octadecatrienoic acid, Ethyl ester (Z,Z,Z), 8,11,14-Eicosatrienoic acid (Z,Z,Z), 5,8,11,14-Eicosatetraenoic acid ethyl ester (all-Z) and Squalene (Table 5).

In vitro anticancer studies

The cytotoxic potency of various extracts was confirmed by *In vitro* cytotoxicity assay methods against animal cancer cell lines and human cancer cell lines. Crude extracts of root bark exudates of *H. binata* in petroleum ether, ethyl acetate, chloroform, methanol and water was obtained separately and they were tested for cytotoxic activity in African green monkey Kidney Epithelial Cells (Vero), Human Cervical Cancer Cell Line (HeLa) and Human Breast Cancer cells (MCF-7) by MTT assay. The cell lines are treated with various extracts of concentrations ranging from 32.25µg/ml to 1000µg/ml. The results in cell growth inhibition by the various extracts and concentrations are shown in Table 6 and Fig. 1.

In Human Breast Cancer cells (MCF-7) IC_{50} value was 193.77µg/ml of petroleum

ether, 213.77µg/ml of chloroform, 210.37µg/ml of ethyl acetate, 234.74µg/ml of methanol and 282.28µg/ml of water. In Human Cervical Cancer Cell Line (HeLa) IC_{50} value was 214µg/ml of petroleum ether, 218.49 µg/ml of chloroform, 241.41µg/ml of ethyl acetate, 282.1µg/ml of methanol and 287.95 µg/ml of water. In African green monkey Kidney Epithelial Cells (Vero), IC50 value was 295.22 µg/ml of petroleum ether, 299.69 µg/ml of chloroform, 317.09 µg/ml of ethyl acetate, 429.7µg/ml of methanol and 312.96 µg/ml of water.

In MCF-7 and HeLa the cytotoxicity activity was found to increase with the polarity of the solvent, i.e. petroleum ether > ethyl acetate > chloroform > methanol > water. The extracts of different solvents have shown cytotoxicity to a certain degree of selectivity against different cell types. The extracts shown a higher degree of inhibition against the Human Breast Cancer cells (MCF-7), Human Cervical Cancer Cell Line (HeLa) in solvents like petroleum ether, ethyl acetate and methanol. The activity may be because of the metabolites present in the crude extracts, which are active against the cell lines. The aqueous extract exhibited only a weak activity against the cell lines tested.

The phytochemical studies indicated the presence of flavonoids, saponins, phenols and tannins. Many such compounds are known to possess potent antitumor properties [15]. The extract of *H. binata* was found to be rich in flavonoids and saponins. Flavonoids have been found to possess anti-mutagenic and anti-malignant effect [16-17]. Moreover, they have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and inhibition of neovascularisation[18]. Saponins have been found beneficial in the inhibition of tumour angiogenesis by suppressing its inducer in the epithelial cells of blood vessels and then on adhering, invasion and metastasis of tumour cells. They also exhibited antitumor effect by cell cycle arrest[19]. The physiological function of five compounds identified in GC-MS were also reported with anticancer properties and they were also reported in different plant species by various authors[20-22].

CONCLUSION

The findings of this study supported the traditional knowledge of Malayali traditional healers and also support folkloric usage of this plant in treating breast cancer. The present study is the first report on the anticancer property of *H. binata.* This study also opens avenues for pharmaceutical researchers to develop a potential anticancer drug.

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REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, *CA Cancer J Clin* 2010; 60:277-300.
2. Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come Pharm Res 2010; 27: 950-61.
3. Desai AG, Qazi GN, Ganju RK, l. Medicinal plants and cancer chemoprevention. Curr Drug Metab 2008; 9: 581-591
4. Krishnaswamy K. Traditional Indian spices and their health significance. *Asia Pac J Clin Nutr* 2008; 17: 265-8.
5. Farnsworth NR .Screening plants for new medicines. In Chapter 9 in Biodiversity.

Edited by Wilson EO. Washington D.C: National Academy Press; 1988.
6. Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer 2002; 2:143–148.
7. Grabley S, Thiericke R. Bioactive agents from natural sources: trends in discovery and application. Adv Biochem Engin Biotechnol 1999; 64:104–154
8. Harbourne JB. Phytochemical methods -A Guide to modern techniques of plant analysis 2nd edition. Chapman and Hall, London; 1984: 4-120.
9. Hebert E, Brain Ellery, Kenneth W. Text book of practical pharmacognosy, Baiilere, London; 1984:363.
10. Wagner H, Bladt S, Zgainski EM. Plant drug analysis, Springer-Verlag, Berlin; 1984: 298-334.
11. Basset JJ, Denny JH, Jeffery J, Mendham. Textbook of Quantitative in organic analysis, 4th edition. ELBS. Longman, Essex, OK; 1985:196.
12. Kokate CK, Parolit AP, Gokhale SB. Pharmacognosy 1st Ed Niralli Prakasan, Pune; 1980; 123.
13. McMaster, Christopher, McMaster, Marvin C. GC/MS: a practical user's guide. New York: Wiley 1998.
14. Altman FP. Tetrazolium salts and formazans. Prog. Histochem. Cytochem. 1976; 9 (3): 1–56.
15. Kintzios SE. Terrestrial plant derived anticancer agents and plant species used in anticancer research. Critical Reviews in Plant Sciences 2006; 25: 79-113.
16. Brown JP. A review of the genetic effect of occurring flavonoids, anthraquinones and related compounds. Mutation Research 1980; 75: 243-77.
17. HiranoT, Oka K, Akiba M. Anti proliferative effects of synthetic and naturally occurring flavonoids on tumour cells of human breast carcinoma cell line, ZR-75-1 Res Commun Chem Pathol Pharmacol 1989; 64: 69-78.
18. Weber G, Shen F, Prajda N. Increased signal transduction activity and down regulation in human cancer cells. Anticancer Research 1996; 16: 3271-82.
19. Man S, Gao W, Zhang Y, Huang L, Liu. Chemical study and medical applications of saponins as anticancer agents. Fitoterapia 2010; 81: 703-14.
20. Zeeshan M, Rizvi SMD, Khan MS, Kumar A. Isolation, partial purification and evaluation of bioactive compounds from leaves of A*geratum houstonianum.* Excli Journal 2012; 11:78-88.
21. Sheela D, Uthayakumari F. GC-MS analysis of bioactive constituents from coastal sand dune taxon – S*esuvium portulacastrum* L. Bioscience discovery 2013;4(1): 47-53
22. Vembarasi G,Velavan S, Mahadevan K. Identification of bioactive components and its biological activities of A*sparagus racemosus* rhizomes through the gas chromatography and mass spectrometry. *International Journal of Natural products research* 2013; 2(1): 17-19.

Table 1. Preliminary phytochemical Screening of the various extracts of root bark exudates of *Hardwickia binata* Roxb.

S. No	Name of the extract	Carbohydrates	Glycosides	Fixed oils & fats	Protein& amino acids	Saponins	Tanins	Phytosterols	Alkaloids	Phenolic compounds	Flavonoids	Gums& Mucilages
1	Petroleum Ether	-	-	+	-	-	-	+	-	-	+	-
2	Chloroform	-	-	-	-	-	-	+	+	-	+	-
3	Ethyl acetate	+	+	-	+	-	+	+	+	-	+	-
4	Methanol	+	+	+	+	+	+	+	+	+	+	-
5	Water	+	+	-	+	+	+	-	-	+	+	-

Table 2. Physico-chemical parameters of root bark exudates Hardwickia *binata* Roxb.

S. No	Treatment	Values
1	Total ash	0.47 g /g dr/wt.
2	Water soluble ash	0.37 g /g dr/wt.
3	Sulphated ash	1.60 g /g dr/wt.
4	Acid insoluble ash	0.27 g /g dr/wt.
5	Ethanol soluble extractive	0.65 g /g dr/wt.
6	Water soluble extractive	0.67 g /g dr/wt.
7	Loss of drying	0.94 g /g dr/wt.

Table 3. Quantitative analysis of root bark exudates *Hardwickia binata* Roxb.

S. No	Name of the Phytochemical content	Values
1	Alkaloids	0.64 g /g dr/wt.
2	Glycosides	0.82 g /g dr/wt.
3	Saponins	0.74 g /g dr/wt.
4	Phenols	1.39 g /g dr/wt.
5	Flavonoids	1.08 g /g dr/wt.

Table 4. Components identified in the root bark exudates of *Hardwickia binata* Roxb.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area
1	6.51	Methoxydi(1-pyrrolidinyl)phosphine	C9H19N2OP	202	0.02
2	7.72	1-tert-Butyl-3-(3-methoxyphenyl)-bicyclo[1.1.1]pentan	C16H22O	230	0.32
3	10.88	Limonene dioxide	C10H16O2	168	1.10
4	11.86	2-[3-Cyclohexylaminopropylamino] Ethylthiophosphate	C11H25N2O3PS	296	0.02
5	12.10	Pentanoic acid, 2-(2-hydroxy-2-methyl-4-phenylbut-3-ynyl)amino-4-methyl-	C17H23NO3	289	2.03
6	12.70	Decanoic acid, ethyl ester	C12H24O2	200	17.55
7	14.27	cis-9-Hexadecenal	C16H30O	238	58.68
8	14.34	4-Octadecenal	C18H34O	266	35.67
9	14.53	N-[3-[6-Hydroxyhexyl]aminopropyl]aziridine	C11H24N2O	200	2.74
10	14.69	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	292	3.91
11	14.95	9,12-Octadecadienoyl chloride, (Z,Z)-	C18H31ClO	298	36.47
12	15.29	Octadecanoic acid, ethyl ester	C20H40O2	312	14.69
13	15.33	4-Hexenoic acid, 2-amino-6-hydroxy-4-methyl-	C7H13NO3	159	0.05
14	15.90	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	C20H34O2	306	2.10
15	16.99	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C20H34O2	306	2.61
16	17.10	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	C22H36O2	332	15.08
17	17.12	2H-Pyran-3-ol, 2-ethoxy-3,4-dihydro-, acetate	C9H14O4	186	0.11
18	18.05	3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino]propionitrile	C14H25N3	235	0.03
19	19.44	Deoxyspergualin	C17H37N7O3	387	0.06
20	19.66	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	C15H26	206	1.38
21	20.03	Benzoic acid, 4-nitro-, 1-methylethyl ester	C10H11NO4	209	0.58
22	23.76	Squalene	C30H50	410	4.78

Table 5. Activity of Components identified in the root bark exudates of *Hardwickia binata* Roxb.

S. No.	Name of the compound	Activity
1	Methoxydi(1-pyrrolidinyl) phosphine	Antimicrobial, Anti-inflammatory
2	1-tert-Butyl-3-(3-methoxyphenyl)-bicyclo [1.1.1] pentan	Activity not reported
3	Limonene dioxide	Fragrance compound
4	2-[3-Cyclohexylaminopropylamino] Ethylthiophosphate	Antimicrobial
5	Pentanoic acid, 2-(2-hydroxy-2-methyl-4-phenyl but-3-ynyl)amino-4-methyl-	Antimicrobial
6	Decanoic acid, ethyl ester	Activity not reported
7	cis-9-Hexadecenal	Antimicrobial, Anti-inflammatory
8	4-Octadecenal	Antimicrobial, Anti-inflammatory
9	N-[3-[6-Hydroxyhexyl]aminopropyl] aziridine	Antimicrobial
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Anti androgenic, Nematicide 5-Alpha reductase inhibitor, Antihistaminic Anticoronary, Insectifuge, Antieczemic **Anticancer**
11	9,12-Octadecadienoyl chloride, (Z,Z)-	Hypocholesterolemic,Nematicide Antiarthritic,Hepatoprotective,Antiandrogenic, Nematicide,5-Alphareductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, **Anticancer**
12	Octadecanoic acid, ethyl ester	Activity not reported
13	4-Hexenoic acid, 2-amino-6-hydroxy-4-methyl-	Antimicrobial
14	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Anti androgenic ,Nematicide 5-Alpha reductase inhibitor, Antihistaminic Anticoronary, Insectifuge, Antieczemic **Anticancer**
15	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	Cardio protective, Hypocholesterolemic Anticoronary ,**Anticancer**
16	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)	Cardio protective ,Hypocholesterolemic Anticoronary, **Anticancer**
17	2H-Pyran-3-ol, 2-ethoxy-3,4-dihydro-, acetate	Antimicrobial
18	3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino]propionitrile	Antimicrobial
19	Deoxyspergualin	Antimicrobial
20	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	Activity not reported

21	Benzoic acid, 4-nitro-, 1-methylethyl ester	Antimicrobial Preservative
22	Squalene	Antibacterial, Antioxidant, **Antitumor, Cancer preventive,** Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide, Diuretic

Table 6. IC_{50} of various extracts against normal and cancer cell lines

S. No	Name of the Extract	IC_{50} (µg/ml)		
		Vero (African green monkey Kidney Epithelial Cells)	HeLa (Human Cervical Cancer Cell Line)	MCF-7 (Human Breast Cancer Cell Line)
1	Petroleum ether	295.22	214.00	193.77
2	Chloroform	299.69	218.49	213.27
3	Ethyl acetate	317.09	241.41	210.37
4	Methanol	429.7	282.1	234.74
5	Water	312.96	287.95	282.28

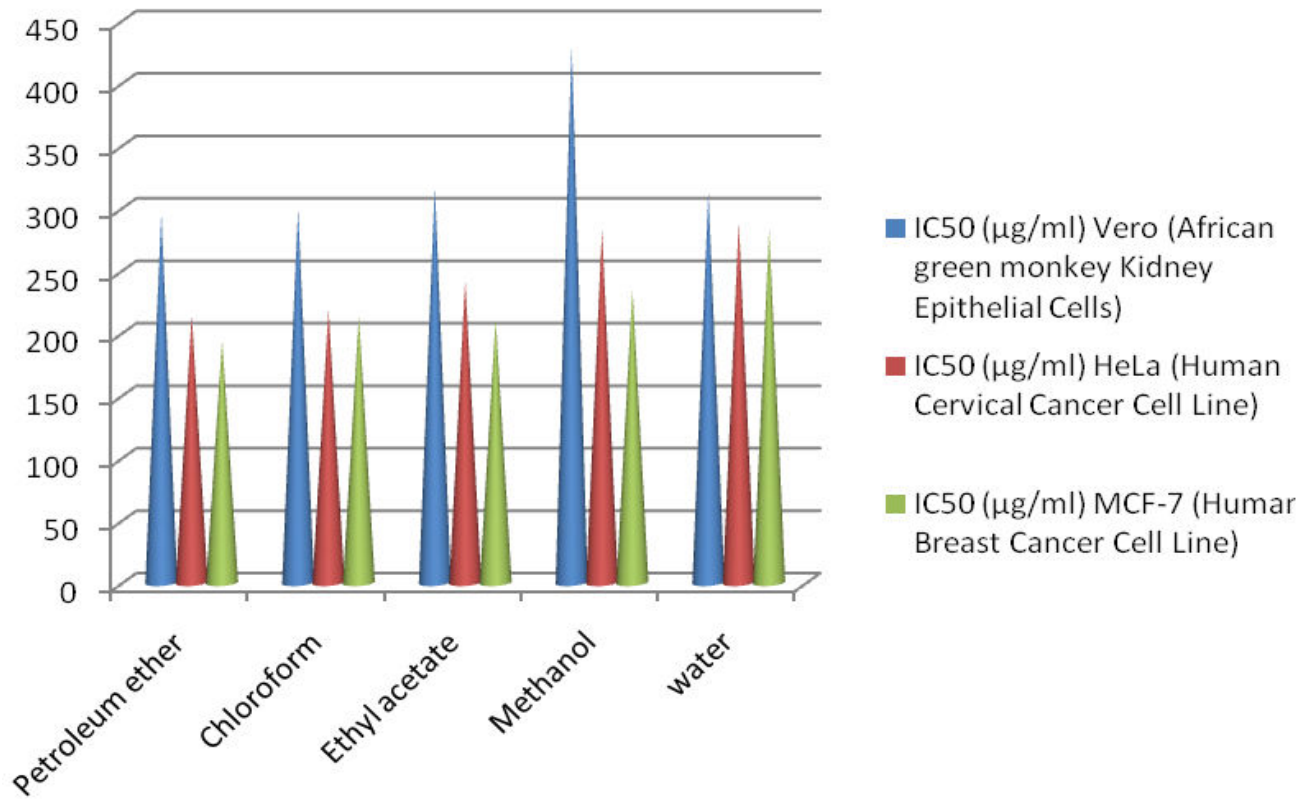

Figure 1. IC_{50} of various extracts against normal and cancer cell lines

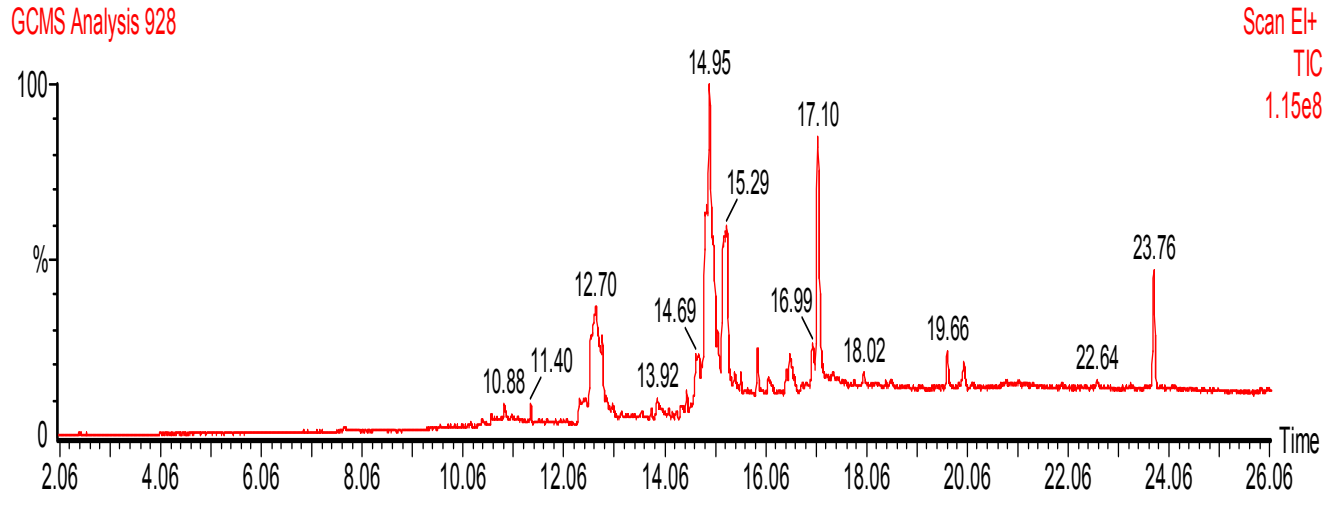

Figure 2. GC-MS Chromatogram of root bark exudates of *Hardwickia binata* Roxb.