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Anti-tuberculosis activity and GC-MS analysis of water extract of *Semecarpus anacardium* nuts

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ABSTRACTS

Semecarpus anacardium (SA) is a high medicinal value plants. The tribal area people has been using nuts of SA for the treating tuberculosis since long time but active chemical constituent are still unknown. Present study was carried out to isolate, identify and evaluate bioactive compounds of SA nuts extracted using Agilent 7890A GC-MS. Solvent extraction of SA nuts was done with petroleum ether, ethyl acetate, methanol and finally with water. All the extracts were tested for their bioactivity against potential pathogen Mycobacterium tuberculosis in collaboration with Central Drug Research Institute, Lucknow, India. The extract which was found to be highly activity in terms of MIC against M. tuberculosis, was subjected for the Gas chromatography and mass spectroscopy. Also potential extract was subjected to phytochemical analysis. Proximate analysis and nutritive value determination was done in SA nuts powder. Also potential extract was subjected to column chromatography over silica gel for fractionations. After sequential elution with distilled water, methanol and their combinations, two fractionations were collected and subjected to TLC for checking proper separation before analyzing by GC-MS. Water extract showed potential with MIC 6.25 µg/ml against M. tuberculosis during in vitro bioassay experiment which is the pointing out towards potential of this plant against this microorganism. Phytochemical analysis results showed the presence of Alkaloids, Carbohydrate, Lipids, Saponins, Flavonoids, Tannins, Sterols and Triterpenes. Total nutritive value in 100g nuts was found 359.2 kilo caloric. In chromatogram of fraction 1 (SA/WE-01), out of six peaks, four peaks were analyzed and identified as per the fragmentation pattern as Benzoic acid 4-ethoxy ethyl ester, 2- lacosanol , n-hexadecanoic acid and n-Heneicosane. In fraction 2 (SA/WE-02) only one peak analyzed and identified as per the fragmentation pattern as Phenol, 2,4-bis (1,1-dimethylethyl). Analysis and identification of presence of compound in these extracts was done using database of NIST library.

Keywords: Bioactive compounds, anti-tuberculosis, Bhillawa, tribal medicines.

ABBREVIATION:
SA = Semecarpus anacardium
GC-MS = Gas Chromatography Mass Spectroscopy
TLC = Thin layer Chromatography
WE = Water Extract
NIST =National Institute of Standard and Technology
TDA =Toluene-diamine
EI = Electron Ionization
PCI = Positive Chemical Ionization
NCI = Negative Chemical Ionization
RT = Retention Time
MF = Molecular Formula

MW = Molecular Weight
amu = atomic mass unit
MIC=Minimum Inhibitory Concentration
CDRI=Central Drug Research Institute

INTRODUCTION

Semecarpus anacardium belonging to family *Anacardiaceae* that usually found in Chhattisgarh, Madhya Pradesh, Himalayas and throughout the hotter parts of India. It is commonly known Bhilawa in Hindi [1]. Various parts of these plants are traditionally being used in Ayurvedic system as drug for alimentary tract and certain dermatologic conditions. Reported constituent in nuts are biflavonoids, biflavone A, biflavone B, biflavone A1, biflavone A2, semecarpuflavone, jeediflavone, tetrahydrobusfltaavone, glluflavone, bhilavinol and reported constituent in leaves are ametoflavone [2,3]. Biomedical action reported are many medical values of this plant are reported in previous work include anticancer, anti-atherogenic effect, anti-inflammatory, antioxidant, antimicrobial, CNS, hypoglycemic, anticarcinogenic, hyperlipidemic activity, heart blood pressure respiration and neurological disorders [4-15]. In Chhattisgarh state, tribal people are using this plant for the treatment of tuberculosis but chemical constituents responsible for the activity are still not properly studied. Objective of the present study is to identify the active chemical responsible for the activity with the aid of GC-MS technique.

MATERIALS AND METHODS

Collection of plant material: Fresh nuts of *Semecarpus anacardium* (SA) (Anacadiaceae) nuts were collected during the month of September 2011 from the tribal area of Ambikapur (Wadrafnagar, GPS: $23^0$45'35.1202"N; Longitude: $83^0$12'18.3107"E) Chhattisgarh India (Plate-I & Plate-II). The plant was taxonomically identified by Professor Dr. KP Sahu, Botany Department Govt. Model Science College Jabalpur.

Extraction of plant material: SA nuts were shade dried and a fine powder was prepared using grinder. 320gm powdered material was extracted with petroleum ether, ethyl acetate, methanol and distilled water. The extracts were concentrated to dryness under reduce pressure by using flask evaporator at controlled 40ºC temperature.

Bioactivity test: Anti-tuberculosis activity of all SA nuts was performed in Lownstein-Jensen medium at CDRI Lucknow against *M.tuberculosis* with two control drugs, Isoniazid and Rifampicin. On the basis of results of bioactivity test, selection of the extract was done for further investigations.

Phytochemical and Proximate analysis: Potential extract was tested for the presence of Alkaloids, Carbohydrate, Lipids, Saponins, Flavonoids, Tannins, Sterols and Triterpenes according to the method described by.

Fractionation: Eight grams of water extract was subjected to column chromatography (1000×40mm) packed with silica gel (100-200 mesh size) in methanol for fractionation. Elute, each of 15ml were collected and continuously monitored by performing TLC to avoid the mixing of fractionating compounds.

GC-MS ANALYSIS OF MATERIAL

Instruments: Agilent 5975C TDA series gas chromatography/mass spectroscopy selective detector system offer high performance and flexibility with many options. Gas chromatograph Agilent 7890A is the auto sampler, oven temperature is ambient +4- 450ºC and 20/21 negative ramps allowed and mass selective detector includes standard mode –EI, optional mode-PCI, NCI and EI acquisition with Cl source, EI Ion source type-non coated inert EI source, Ion source temperature-150^0C to 350^0C, Quadrupole temperature-106-200^0C, mass filter-monolithic hyperbolic quadrupole, minimum mass-1.6u, maximum mass-1050u, mass axis stability- better than 0.10u/48h, detector- triple axis.

Method of GC-MS analysis and chromatographic condition: GC/MS make Agilent (5975CMS) was used for the identification of constituents with 5% poly siloxane column 30×250µm×0.25µm size at Indian Institute of Science Research and Education, Bhopal. Oven temperature was programmed as Isothermal temperature was 5ºC/min and held for 1.75 min then increased to 275ºC at the rate of 8ºC/min and kept constant for 5min. The run time was 25 min. Ionization of sample components were performed on EI mode (70eV).

Identification of bioactive compounds: Identification of bioactive compounds and interpretation of mass spectrum (GC-MS) was conducted using the database of National Institute of Standard and Technology (NIST). The name, structure and molecular weight of the compounds in sample material were ascertained.

RESULTS

Bioactivity of water extract of SA nuts against *M. tuberculsis* screened was found best as MIC value was calculated as 6.25µg/ml, ($F_{(P<0.01)}$ =120.96, *df*=10, $SE_{(d)}$± 0.0001, $LSD_{(P<0.05)=}$ 0.00013) **Table-3** which was significantly different from other extracts thus selected for the further chemical analysis. Photochemical chemical analysis results of water extract showed the presence of Alkaloids, Carbohydrate, Lipids, Saponins, Flavonoids, Tannins, Sterols and Triterpenes are listed in **Table-2.** Results of proximate analysis of SA nuts powder are summarized in **Table-3**. By the column chromatography, two fractions of water extracts were collected. In gas chromatogram of water extract fraction 1 (SA/WE-0; Fig 1), six peaks were obtained. Out of six peaks, four peaks could be analyzed and identified as per the fragmentation pattern as Benzoic acid 4-ethoxy ethyl ester with RT 10.79 min. and 6.80% peak area (Fig 2); 2- lacosanol with RT 14.04 min. and 6.25% Peak area, (Fig 3); n-hexadecanoic acid with RT 14.28 min. and 12.00% peak area (Fig 4) and n-Heneicosane with RT 15.92 min. and 6.33% peak area (Fig 5). In fraction 2 (SA/WE-02; Fig 6) only one peak could be analyzed and identified as per the fragmentation pattern as Phenol 2, 4-bis (1,1-dimethylethyl) with RT 10.62 min. and 13.59% peak area (Fig 7) was confirmed. All the compounds identified are listed in **Table-4.**

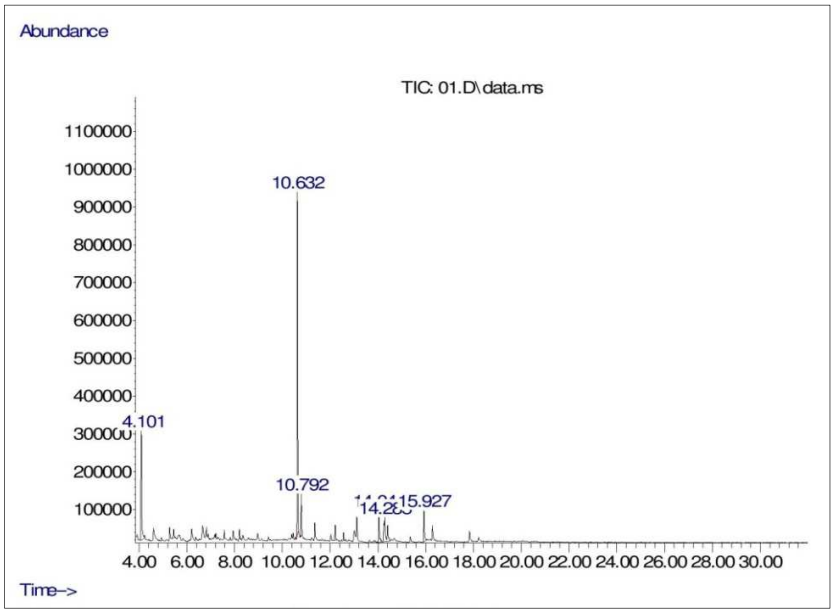

Figure 1 Gas chromatogram of SA/WE-01

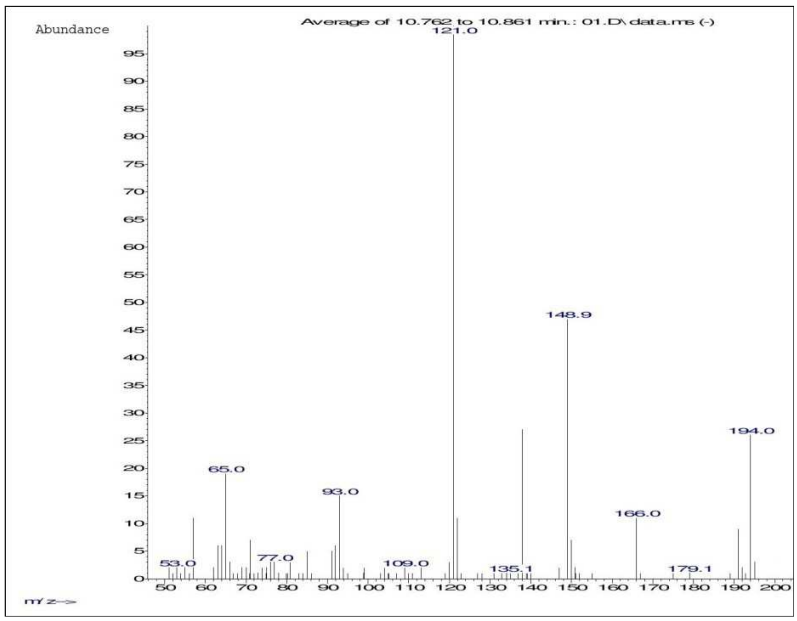

Fig 2: MS of Benzoic acid, 4-ethoxy ethyl ester

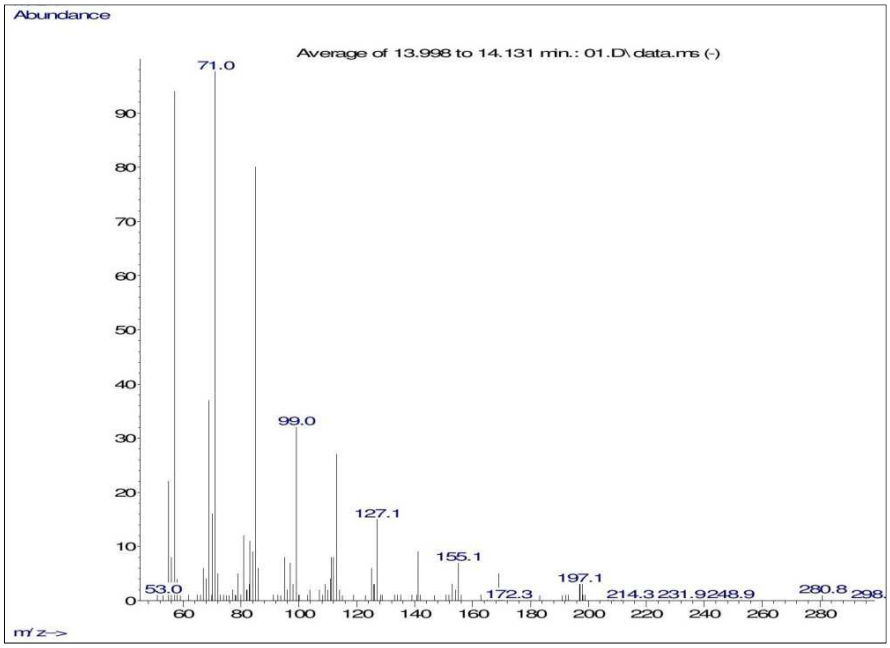

Fig 3: MS of 2- Lacoansol

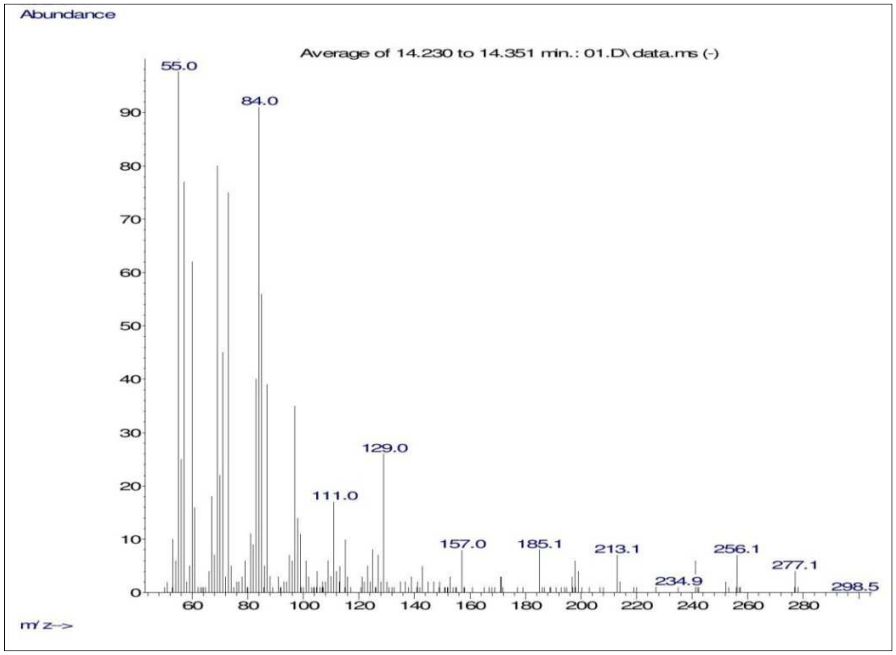

Fig 4: MS of Hexadecanoic acid

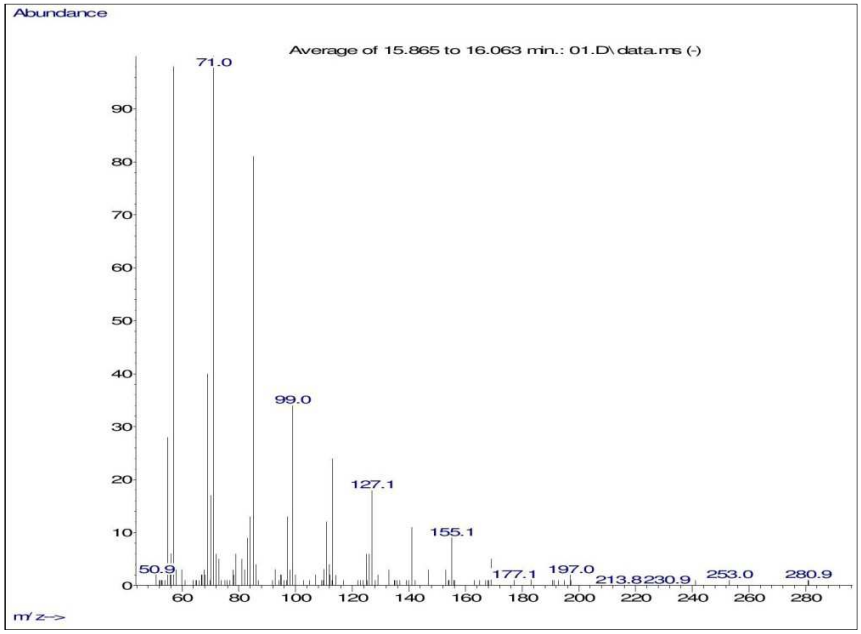

Fig 5: MS of n-Heneicosane

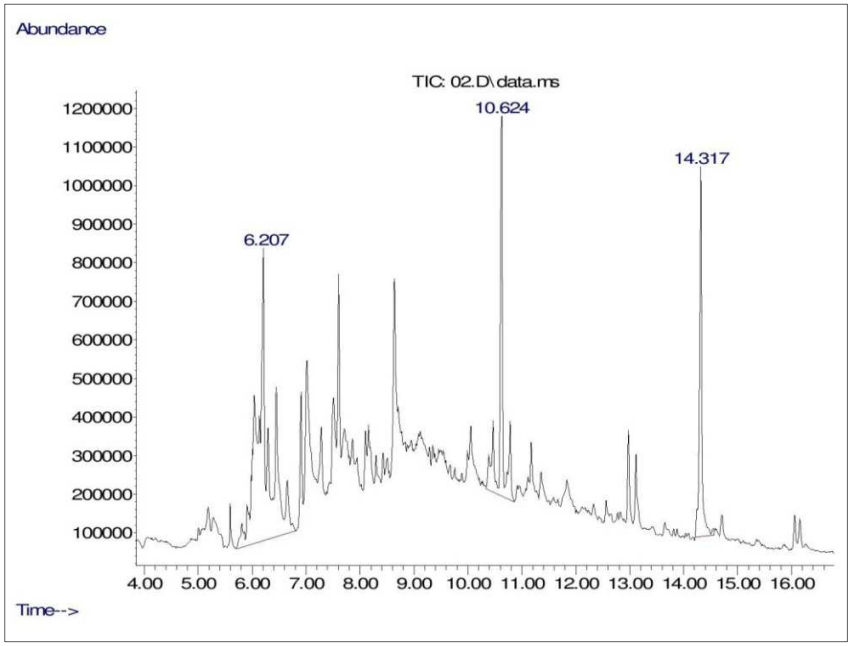

Fig 6: Gas chromatogram of SA/WE-02

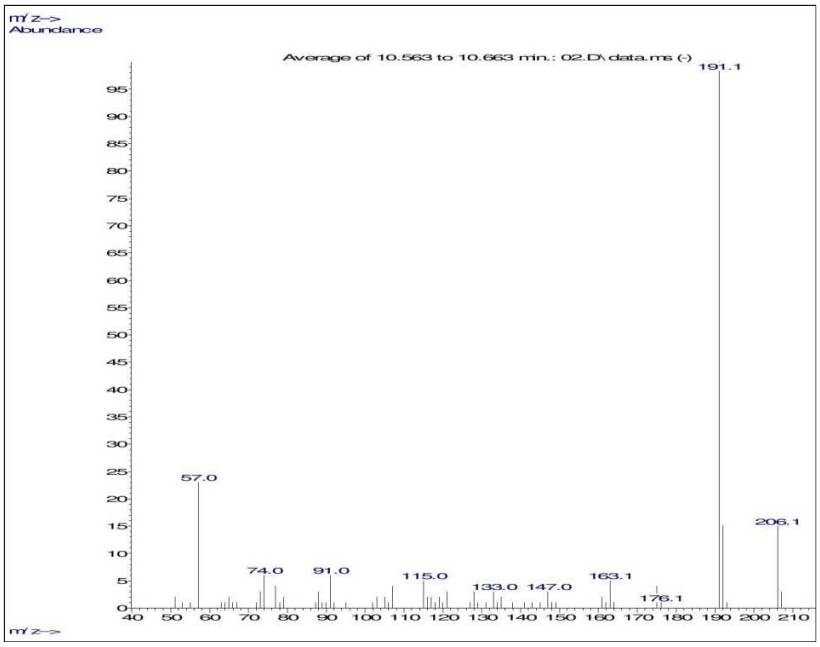

Fig 7: MS of Phenol 2, 4-bis (1, 1-dimethylethyl)

Table 1: Phytochemical analysis of SA nuts extract in water after serial extraction

S. No.	Qualitative tests	Results
1.	Alkaloids	+
2.	Carbohydrarte	+
3.	Protein	-
4.	Lipids	+
5.	Saponins	+
6.	Flavinoids	+
7.	Resins	-
8.	Tannins	+
9.	Sterols	+
10.	Cardiac glucosides	-
11.	Triterpenes	+
12.	Coumerins	-
13.	Anthraquinone	-

Table 2: Proximate analysis and nutritional values of SA nuts powder

S. No.	Components	Plant material (SA nuts powder)
1	Protein %	19.8
2	Fat %	14.0
3	Fiber%	7.4
4	Carbohydrate%	38.5
5	Ash%	10.8
6	Moisture%	8.9
7	Nutritive Value (Kcal per 100 g)	359.2

Table 3: Bioactivity of SA nuts extracts against *M. tuberculosis*

Treatments	MIC of plant extract (In µg/ml)
	SA (Nuts Extract)
PEE	12.50^{d}
EAE	$>50.0^{f}$
ME	25.0^{e}
WE	6.25^{c}
Isoniazid	0.05^{a}
Rifampicin	0.2^{b}
$F_{(P<0.01)}$	120.96
df	10
$SE_{(d)}\pm$	0.0001
$LSD_{(P<0.05)}$	0.00013

Table 4: Phytochemicals identified in SA nuts extract in water

Fraction No.	S. No.	RT (Min.	Compounds Name	Molecular Formula	M.W. (amu)	Peak % area
1	1	10.79	Benzoic acid,4-ethoxy ethyl ester	$C_{11}H_{14}O_3$	194	6.80
	2	14.04	2- Lacoansol	$C_{20}H_{42}O$	298	6.25
	3	14.28	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.00
	4	15.92	n-Heneicosane	$C_{21}H_{44}$	296	6.33
2	5	10.62	Phenol 2,4-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	206	13.59

DISCUSSION

Anti-tuberculosis activity of this plant extract is previously not reported in available literature so the results cannot the compared with any report for its activity against *M. tuberculosis*. However other plants, *Alatonia scholaris* [16], *Marrubium vulgare* [17] *Azadirecta indica* [18] and *Anogeissus leiocarpus* [19] are reported for their potential against the deadly disease, tuberculosis. Although whole spectrum could not be completely analyzed but what done was surprising in results. Previously no results discussed in any literature about the chemical composition of SA nuts but the presence of phenolic compounds, bhilavanol A (monoenepentadecyl catechol 1), bhilavanol B

(dienepentadecyl catechol II) and anacardoside are reported [20]. Important biflavanoids such as semecarpuflavanone, jeediflavanone, galluflavanone, nallaflavanone, semecarpetim and anacarduflavanone but no compound was found similar with the compound identified in nuts extract of present study. Nuts extract showed antituberculosis activity during in vitro bioassay investigations but particular compound responsible for this activity could not be pointed out and will be the extension of the present work. In water extract of SA nuts, only five compounds could be identified but most of the peaks are unidentified indicating further scope for the identification of more compounds and their particular activity.

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