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Investigation of chemical composition of methanolic extract of *Anisomeles indica* (L.) Kuntze by using FTIR and GC-MS

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Abstract
Introduction: *Anisomeles indica* (L.) Kuntze (Lamiaceae) is camphor scented perennial weed have brush leaves. Traditionally, the plant has been used as folk medicine to cure fever, whooping cough, uterine infection, allergy, snake bite, liver protection, mouth abscess and toothache.
Method: Plant extract from aerial parts of *A. indica* prepared in Soxhlet assembly. Screening for phyto chemicals was carried out as per standard methods. FTIR and GC-MS analysis of the methanolic extracts of *A. Indica* was performed.
Results: FTIR results find out the major peaks between ranges of 952 cm^{-1} to 3430 cm^{-1}. FTIR peak spectrum indicated the presence of alcohols, alkanes, alkyl halides, unsaturated hydrocarbons, fatty acids, amines, acid anhydride and carboxylic compounds. GC-MS chromatogram of methanolic extract showed the presence of 14 phyto compounds. Out of 14 compound, three compounds have high percentage of Cyclohexane,1-ethnyl-1-methyl-2,4-bis(1-methylethyl)-[1S-(1-alpha,2-bet-(21.98), Caryophyllene (27.68), Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-diemthyl-2-methylethenyl) (25.98) and the low percentage of Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene(0.406).Mainly terpenoids have been detected from the methanolic extract of this plant most of which are sesquiterpenes. Others are alkaloids and phenols. Sesquiterpenes display antimicrobial, anti-inflammatory, and anti-cancerous property.
Conclusion: Present investigation reveals the presence of bioactive compounds in the methanolic extract, which have of phyto pharmaceutical significance.

Keywords: *A. indica*, Methanolic extract, Phyto compounds, FTIR, GC-MS

Introduction
Plants are the store house of various natural bioactive compounds, effectively have biological activities. Traditionally plants are used for health care. Bioactive compounds are secondary metabolites such as monoterpenes, flavonoids, sesquiterpenes, alkaloids, saponins, phenols, glycosides mainly used in food, pharmaceutical, chemical, cosmetic industries, and agriculture [1-2]. Lamiaceae family is aromatic compound family, rich in phenolic derivatives, terpenoids, which have medicinal importance. *Anisomeles indica* (L.) Kuntze is weed belongs to Lamiaceae family, popularly known as Indian Catmint, grow in the temperate and tropical region of Asia, including in India [3]. It is prevalent dominant in north and northeast India grows along the margins of agriculture and wasteland, especially during the season of March to October. The Entire plant has medicinal significance especially, Aerial parts and leaves evacuate pungently, Camphor scented odor [3-4]. Traditionally the plant has been used as a folk medicine to cure fever, whooping cough, uterine infection, allergy, snake bite, mouth abscess, toothache [5-6]. A previous studyhas shown that different extract of *A. indica* possesses antioxidant, anti-inflammatory, antibacterial activity and anti-hype ralgesic, analgesic properties [7-8-9-3-10]. Itcontains secondary metabolites such as flavonoids, diterpenoids, phenyl propanoids, and steroids [11-12]. Previous s investigation reports that *A. indicahas* different types of constituents such as, flavonoids, steroids, arachnoids, alkaloids, terpenoids [13-14-15-16-17-18-19-20]. Characterization revealed the presence of phyto compound such as aniso melic acid, 4-7-oxycycloanisomelic acid, iso-ovatodiolide, stigmasterol and sitosterol [21-22]. However, further research is requisite to find out phyto active compounds by FTIR and GC-MS.

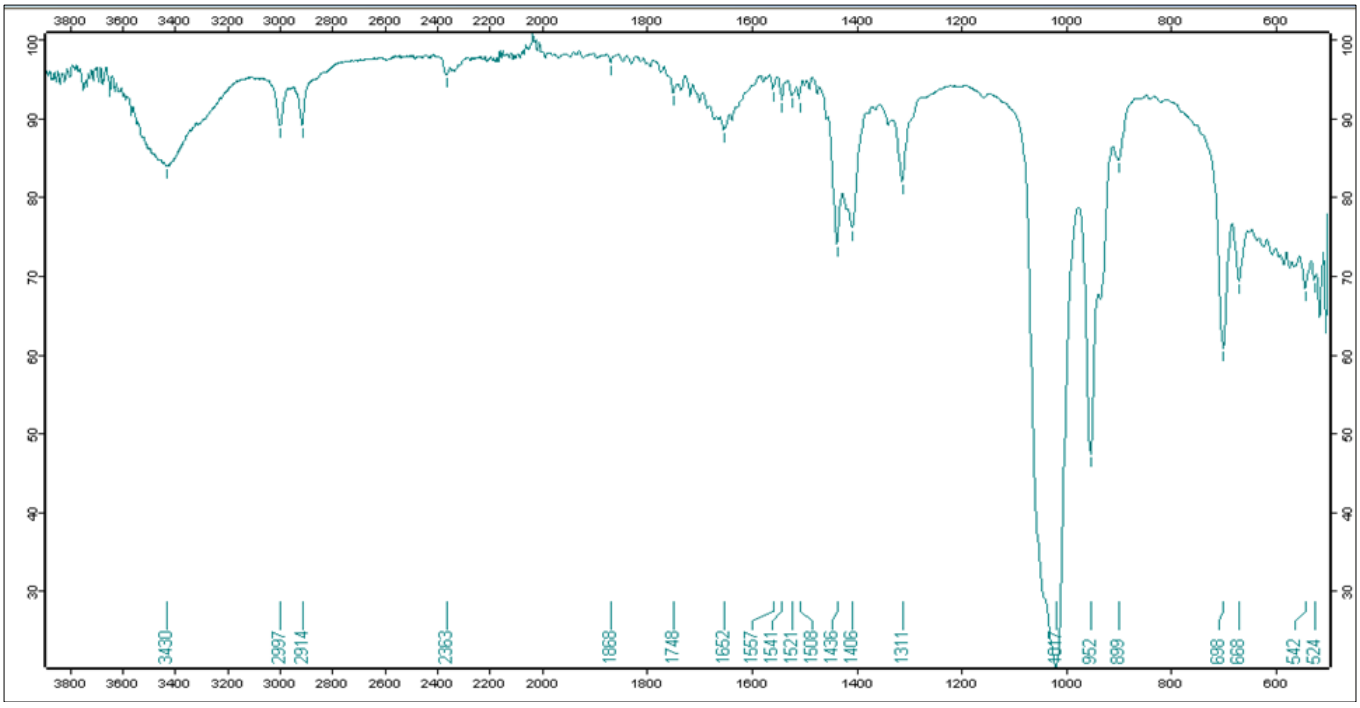
Materials and Methods
Preparation of extract for GC-MS:
Fresh aerial vegetative parts of *A. indica* were collected from Samar Gopalpur village near Rohtak, Haryana, India in the month of April, 2018. Collected plant material was washed with dH_2O and dried at room temperature.

The dried material of plant was grinned to a fine powder. Powdered material (50 gm) was extracted (1:5 W/V) in a Soxhlet apparatus with methanol (250 ml, Merck) till the solution becomes clear. The plant extract was filtered through a what man paper no 1 and then concentrated by using a rotary evaporator (Buchan make) at low temperature. This extract obtained was used for further analysis.
The extracts were stored at 4°C for further uses.

FTIR Analysis
Fourier Transform Infrared Spectro photometer (BRUKER, ALFA MODAL, ABS /Transmittance) with a wavelength range of 4000-400 cm-1with a resolution of 4 cm-1was used to find out the functional groups of compounds present in the methanolic extract by absorbing light in the infra-red region. The methanolic extract of the plant was directly loaded in FTIR spectrometer. The FTIR generate the spectrum of functional groups by measuring the vibration of bond

GC-MS Analysis
Extract of *A. indica* in methanol was exposed for phytochemical analysis using GCMS analyzer (BRUKER SCION 436-GC SQ). Extracts were dissolved in methanol (HPLC grade, Hi Media Laboratories Pvt. Ltd. India) and filtered by what man TMFILTER DEVICE (0.2 µm). 99.99 % of helium gas was used as carrier, with flow rate of 1 ml/min. RESTEK Rtx®-5 [Cross bond® with 5%, biphenyl and dime thicone (l/95%)] with 30metre length, 0.25 µmdf and 0.25 mm ID column was used for separation of phytochemicals with injected temperature 280°C. 2 µl of sample was injected in the column. Initial temperature of oven is 70 °C that hold for 2 min. and slowly raised the temperature with 7 °C rate per minute up to 320 °C and Ion source temperature was sustained at 250 °C. Spectrum was obtained at 70eV by electron ionization and the detection of compound by detector conduct in mode of scan (30–500 Da atomic units). 38.71 min including 3 min solvent delay was total running time. The phytoconstituents identification was verified based on the relative retention time.

Results and Discussion
FTIR Analysis
The FTIR absorption spectrum of methanolic extract were was shown in Figure1 and table 1. Major peaks in absorption were observed at 698 cm^{-1} (C=C stretching as alkanes), 952 cm^{-1} (C-O stretching), 1017 cm^{-1} (C-O stretching as alcohol), 1311 cm^{-1} (C-N stretching as amine), 1406 cm^{-1} (C-O stretching as alcohol), 1436 cm^{-1} (C-H stretching as alcohol), 2914 cm^{-1} (-CH3- as alkanes), 2997 cm^{-1} (-CH3- as alkanes), and 3430 cm^{-1} (N-H stretching as amine). The peaks at 524 cm^{-1},542 cm^{-1} represented C-Cl as halogen, 899 cm^{-1}, 952 cm^{-1} assigned C=C alkenes. Spectrum at 1521 cm^{-1}, 1652 cm^{-1} indicated the aldehyde groups. The peak at 1557 cm^{-1} assigned as amines and other peaks represented 1508 cm^{-1}, 1541 cm^{-1}, C=C stretching (Aromatic rings), 1748 cm^{-1} acid anhydride, 1868 cm-1 acid anhydride, 2363 cm^{-1} alkyne groups. The spectrum of all these peaks indicated the presence of alcohols, alkanes, alkyl halides, unsaturated hydrocarbons, fatty acids, amines, acid anhydride and carboxylic compounds that are present in secondary metabolites such as flavonoids having -OH group, -C=O group, phenolic compounds having –OH functional groups.



Graph 1: Representation of peak value of methanolic extract of *A. indica* by FTIR

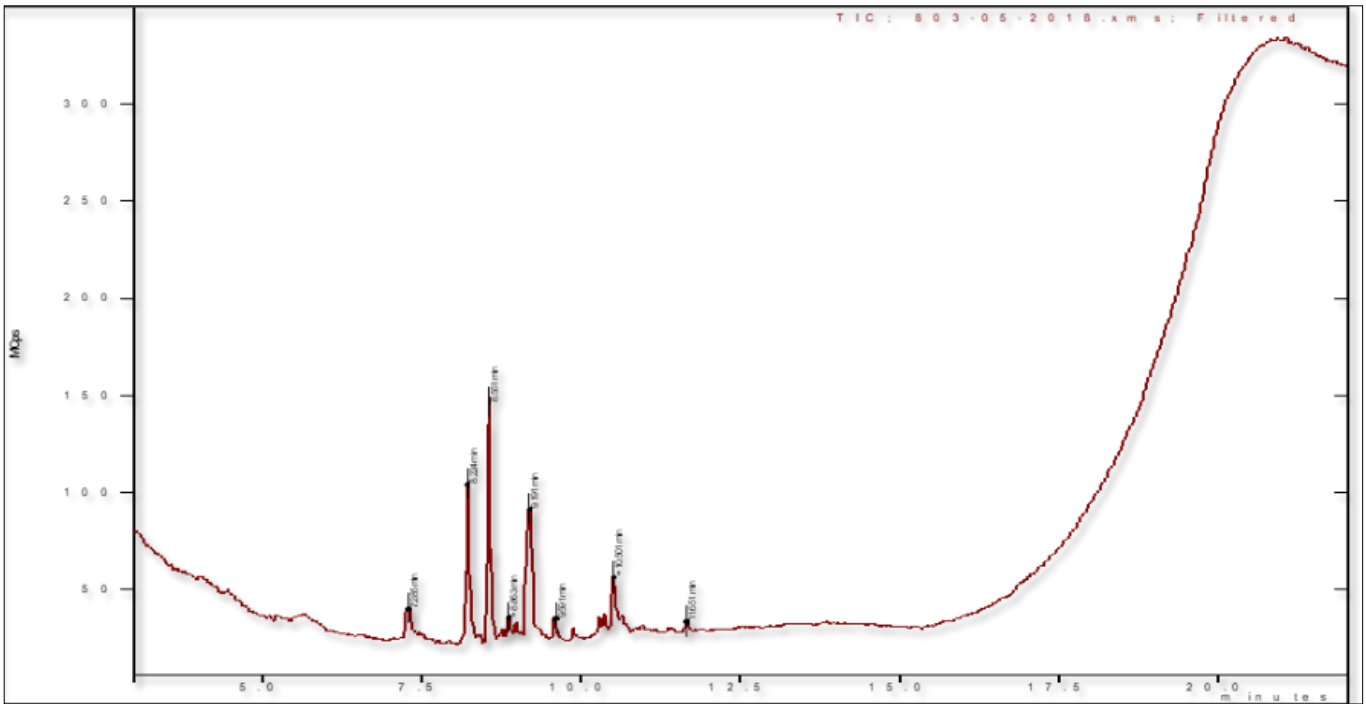
Table 1: Represented FTIR analysis of methanolic extract of *A. indica* with peak value, stretching and functional groups.

524	C-X (Chloride)	Halogen
542	C-X (Chloride)	Halogen
668	C=C Stretching	Alkenes
698	C=C Stretching	Alkenes
899	C=C Stretching	Alkenes
952	C=C Stretching	Alkenes
1017	C-O stretching	Alcohol

1311	C-N stretching	Amines
1406	C-O stretching	Alcohol
1436	C-H stretching	Alcohol
1508	C=C stretching	Aromatic rings
1521	C-H stretching	Aldehyde
1541	C=C stretching	Aromatic rings
1557	N-H stretching	Amines or amide
1652	C-H stretching	Aldehyde
1748	C=O stretching	Acid anhydride
1868	C=O stretching	Acid anhydride
2368	C=C stretching	Alkyne
2914	-CH_3- stretching	Alkanes
2997	-CH_3- stretching	Alkanes
3430	N-H stretching	Amines and amide

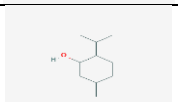
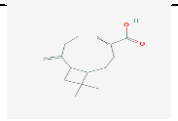
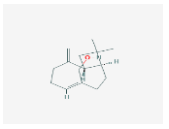
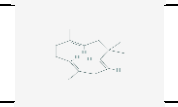
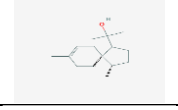
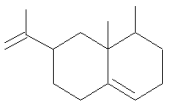
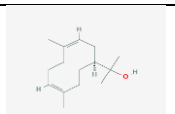
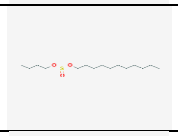
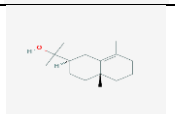
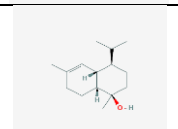
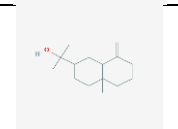
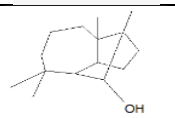
GC-MS analysis

GC-MS chromatogram of methanolic extract was showed the presence of total 14 compounds with their respective area, retention time, percent of total, molecular weight, molecular formula, structure of compound with their nature and biological functions (Figure 2 and Table-2). The spectrum of all phyto compounds present in methanolic extract that were matched with NIST library in the X calibur software of GC-MS apparatus. Out of 14 compound, three compounds have high % of total that were Cyclohexane,1-ethnyl-1-methyl-2,4-bis(1-methylethyl)-[1S-(1-alpha,2-bet-(21.98), Caryophyllene (27.68), Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a, 8-diemthyl-2-(1-methylethenyl)-(25.98). Caryophyllene is type of Sesquiterpene that were helpful to reduce pain, inflammation and also have antibacterial, antifungal properties [23]. Other compounds such as Cyclohexasiloxane, dodecamethyl- (5.262), 1,4-Methanozulen-9-ol,decahydro- (5.494) Humulene(3.116), 3,7-Cyclodecadiene-1-methanol,alpha, alpha, 4,8-tetramethyl-[S-(Z,Z)] (2.15), have moderate % of total, whereas Alpha-acorenol (2.00), Tau. Muurolol (1.469), Sulfurous acid, butyl undecyl ester (1.275), Gama. endesmol (1.203), 1,4-Methanozulen-9-ol,decahydro- (1.086), Cyclohe ptasiloxane, tetrad ecamethyl (0.874), Bicyclo [7.2.0]. Undec-4-ene, 4,11,11-trimethyl-8-methylene(0.406), Cyclo hexasiloxane, dodecamethyl- used in cosmetics to care of Skin, also have antimicrobial property. Bicyclo [7.2.0]. Undec-4-ene, 4,11,11-trimethyl-8-methylene is type of sesquiterpene have anti-inflammatory effect. Humulene is type of Caryophyllene have antimicrobial and anti-cancerous property [24, 25]. Alpha -acorenol act as odorant in essential oils, also inhibit growth of bacteria and fungus [26]. Muurolol also type of sesquiterpene hydrocarbon, showed antifungal activity. Other compound functions were not reported in previous studies.



Graph 2: Graphical representation of GC-MS analysis of methanolic extract of *A. indica*.

Table 2: Elucidation of phytocompounds of methanolic extract of *A. indica* with molecular weight, molecular formula, and structure by qualitative GC-MS analysis

Name of compound	Area	% of Total	Molecular Weight	Molecular Formula	Structures (***)	Compound nature	Biological functions
1.Cyclohexasiloxane, dodecamethyl-	9.012e+7	5.262	444	$C_{12}H_{36}O_6Si_6$		Cosmetics	Skin care, cosmetics products, breast implantation, Antimicrobial [27]
2.Cyclohexane,1-ethnyl-1-methyl-2,4-bis(1-methylethyl)-[1S-(1-alpha,2-bet-(28)	3.765e+8	21.981	204	$C_{15}H_{24}$		Unknown	Unknown
3.Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene	6.957e+6	0.406	204	$C_{15}H_{24}$		Sesquiterpene	**Anti-inflammatory effect
4. Caryophyllene	4.741e+8	27.680	204	$C_{15}H_{24}$		Sesquiterpene	**Treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, and fungal and bacterial infections [23]
5. Cycloheptasiloxane, tetradecamethyl	1.496e+7	0.874	518	$C_{14}H_{42}O_7Si_7$		Cosmetics	Antimicrobial activity [27]
6.Humulene	5.341e+7	3.118	204	$C_{15}H_{24}$		Terpene	**Antibacterial, antifungal and anti-cancerous activity [24, 25]
7. Alpha-acorenol	3.433e+7	2.004	222	$C_{15}H_{26}O$		Sesquiterpene	**Act as odorant inessential oils, Antibacterial activity, Aniinflammatory [29]
8.Naphthalene,1,2,3,4,4a,5,6,8a octahydro-4a,8-diemthyl-2-(1-methylethenyl)- [30]	4.451e+8	25.988	204	$C_{15}H_{24}$		Unknown	unknown
9.3,7-Cyclodecadiene-1-methanol,alpha, alpha, 4,8-tetramethyl-[S-(Z,Z)]	3.698e+7	2.159	222	$C_{15}H_{26}O$		Unknown	Unknown
10. Sulfurous acid, butyl undecyl ester [31]	2.184e+7	1.275	292	$C_{15}H_{32}O_3S$		Unknown	Unknown
11.Gama.eudesmol [29]	2.061e+7	1.203	222	$C_{15}H_{26}O$		Unknown	Unknown
12. Tau. Muurolol [32]	2.516e+7	1.469	222	$C_{15}H_{26}O$		Sesquiterpene	**Antifungal activity
2-Napthalene methanol,dechydro-alpha alpha,4a-trimethyl-8-methylene-[30, 33]	9.416e+7	5.494	222	$C_{15}H_{26}O$		Unknown	Unknown
14.1,4-Methanozulen-9-ol,decahydro- [30]	1.860e+7	1.086	222	$C_{15}H_{26}O$		Unknown	Unknown

* RT- Retention time; Area; % of Total; ** Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database], ***Structure obtained from Pub Chem

Conclusion

GC-MS profiling of *A. indica* reveal that methanolic extract have diversified terpenoids, specially sesquiterpenes, monoterpenes and alkaloids. These compounds have their

medicinal importance that can encourage to find out new therapeutic uses in health care system. On the other hand, isolation and screening of individual phyto-compound is required for their further activity.

Abbreviations Used

A. indica-Anisomeles indica, FTIR- Fourier-transform infrared spectroscopy, GC-MS- Gas chromatography mass spectrometry

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