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Comparative Analysis of Ethanolic Juniperus Thurifera Leaf, Stem Bark and Root Extract Using Gas Chromatography and Mass Spectrometry

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Abstract: ***Medicinal plants are reservoir of bioactive compounds or phytochemicals (terpenoids, alkaloids, tannins, flavonoids and so on) with marked pharmacological properties such as; antimicrobial, antioxidant, anti-inflammatory, antibacterial, analgesics, antiprotozoal, cytotoxic, anti-androgenic, antiviral, antipyretic, antitumor, anti-depressant, hypolipidemic and antihelminthic activities. This study was designed to examine the comparative analysis of ethanolic Juniperus thurifera leaf, stem bark and root extract using gas chromatography and mass spectrometry. Result obtained shows that Juniperus thurifera leaf extract contained 33 bioactive compounds which accounts to 75.47 % while Juniperus thurifera stem bark and root extract contained 37 and 50 phytochemicals with an aggregate of 80.77 % and 93.07 % respectively. Monoterpenes hydrocarbon (40.50 %), oxygenated monoterpenes (12.66 %), sesquiterpenes hydrocarbon (13.79 %) and oxygenated sesquiterpenes were found in Juniperus thurifera leaf extract while Juniperus thurifera stem bark and root extract contains monoterpenes hydrocarbon (48.72 %, 51.33 %), oxygenated monoterpenes (14.88 %, 20.49 %), sesquiterpenes hydrocarbon (10.71 %, 14.25 %) and oxygenated sesquiterpenes (6.56 %, 7.88 %) respectively. It was concluded that Juniperus thurifera leaf, stem bark and root ethanolic extract contains several secondary metabolites which can be used for the treatment of diseases without causing any deleterious effect on the environment and general performance of animals.***

Keywords: ***Gas Chromatography, Mass Spectrometry, Juniperus Thurifera, Leaf, Root, Stem Bark, Phytochemicals***

1. INTRODUCTION

Medicinal plants are a rich source of phytochemicals and natural compounds (alkaloids, flavonoids, steroids, terpenoids, tannins and phenols) with marked therapeutic properties (Ruchika *et al*., 2022; Roma-Marzio *et al*., 2017). They are potential source of drugs and are

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traditionally used in the treatment of digestive disorders, measles, cold, cough, diarrhea, arthritis, skin disease, joint pain, hemorrhoids, fever, sore throat and sexually transmitted infections (Misharina *et al*., 2009; Satrani *et al*., 2019). Medicinal plants are safe, environmentally friendly and performs antioxidant, antifungal, antiprotozoal, cytotoxic, hepato-protective, antibacterial, angelsics antipyretic, anti-inflammatory, antipyretic, antihelminthic, anti-fibrotic, hypolipidemic, anti-tumor and antioxidant activities (Cosentino *et al*., 1999; Alessandra *et al*., 2005).

There are over 200,000 species of medicinal plant with several pharmacological properties and many are still yet to be discovered (Alagbe, 2022). Unlike most synthetic or conventional drugs, medicinal plants are used to boost the immune system, suppress the activities of pathogenic organisms and maintain the general health status of animal without causing any deleterious effect (Olafadehan *et al*., 2021). In the midst of the prospective medicinal plant is Juniperus thurifera which is reported to contain several secondary metabolites necessary to bridge the gap between livestock production and food safety (Shittu et al., 2021). Juniperus thurifera is an evergreen multipurpose small coniferous tree belonging to the family capricidae and class pinopsida. It is native to South Western Europe, North Africa and some parts of Asia including India (Farjon, 1992). The trees can grow up to 20 m tall with single or multiple trunks. The leaves are light green while the stems are grey brown at maturity with several bioactive compounds such as; α-Murolene, α-Longipinene, γ-eudesmol, β-Cayrophyllene, α-Pinene, Torreyol-α-cadinol, α-Cadinol, α-Thujone, β-Phellandrene, Cis-thujanol and many more (Barrero et al., 2004). In vitro analysis has shown that ethanolic and methanolic extract from Juniperus thurifera leaf, stem bark and root can suppress the activities of some Gram +ve and –ve bacteria's such as; Salmonella spp, Staphyllococcus spp, Klebsiella spp, Micrococcus luteus, Escherichia coli, Citrobacter freundii, Bacillus substilis, Neisseria spp and Enterobacter spp thus preventing dysbiosis (Monsouri et al., 2010; Hajjouji et al., 2019).

The use of gas chromatography and mass spectrometry will further help to identify some trace elements, gases as well as giving a clue on possible contaminants in the sample. Therefore, this experiment was designed to examine the comparative analysis of ethanolic *Juniperus thurifera* leaf, stem bark and root extract using gas chromatography and mass spectrometry.

Site of the experiment
The experiment was carried out at the Department of Microbiology, Sumita Research Institute located within 23º 13'N 72º41'E Gujarat India in the month of September to November, 2022.

Collection of plant material and extraction methods
Fresh *Juniperus thurifera* leaf, root and stem bark were harvested in Punsar village Gujarat. It was identified and authenticated by a Taxonomist (Dr. Singh Amit). It was washed separately with running tap water chopped into bits and allowed to dry under shade for 25 days. The dried samples were grinded separately using an electric blender. Dried *Juniperus thurifera* leaf, stem bark and root were soaked separately in ethanol (70 %) over a 48 hours period at a room temperature of 25**ºC** with soxhlet apparatus. The mixture was stirred and filtered with

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Whatman filter paper, thereafter, it was dried in a rotary evaporator. The extract was stored individually a well labelled container and kept in the refrigerator at 4 **ºC** before it was sent to the laboratory for additional examination. JTLE (Juniperus thurifera leaf extract), JTSBE (Juniperus thurifera stem bark extract) and JTRE (Juniperus thurifera root extract).

Analysis of bioactive compounds in *Juniperus thurifera* leaf, stem bark and root extract

Secondary compounds in *Juniperus thurifera* leaf, stem bark and root extract were carried out using Labtron high precision Gas chromatograph mass spectrometer (Model GC-MS-879) with pre-filter mass analyzer and electron multiplier ensuring high sensitivity. Desorption of the extracts were performed at a temperature between 100 – 350 ºC with a column flow rate of 1.0 -1.5 mL per minute with slit ratio of 1000: 1, pressure range (0-100 psi), temperature programming (7 stages/ 8 platforms) and electronic pressure control which supports CV and CC.

Table 1: Bioactive compounds in Juniperus thurifera leaf, stem bark and root extract using GC-MS

Bioactive compounds	**% Area**	**% Area**	**% Area**
	JTLE	**JTSBE**	**JTRE**
γ-Gurjunene	5.72	0.15	1.93
β-Eudesmol	-	1.84	0.60
α-Cadinol	0.01	0.06	1.37
α-Humulene	1.12	1.51	2.05
δ-Cadinene	0.40	0.37	0.21
α-Cubebene	-	-	1.55
γ-Cadinene	0.05	0.83	0.78
Germacrene D	-	0.20	1.49
Sabinene	15.88	17.03	20.82
γ-Terpinene	0.01	0.88	1.53
α-Longipinene	4.02	5.06	1.07
α-Murolene	-	1.41	0.59
γ-eudesmol	0.07	1.05	0.70
β-Bourbonene	-	-	1.22
β-Cayrophyllene	1.76	2.09	0.15
β-Santalene	-	-	1.00
α-Pinene	16.81	19.97	24.31
β-Citrylideneethanol	0.01	0.06	1.50
Torreyol-α-cadinol	0.03	0.01	0.07
D-Limonene	3.08	4.55	2.01
Elemol	-	-	0.01
Germacrene B	-	-	0.02
α-Cadinol	0.05	0.67	0.46
δ-2- Carene	0.93	0.55	1.01
δ-3- Carene	0.92	-	0.13
α-Pinene oxide	1.00	1.88	0.02
(Z)- β- Ocimene	0.02	-	0.25

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δ-Cadinene	0.57	1.21	1.40
β-Eudesmol	0.61	0.40	1.22
Thymol	0.25	1.22	0.05
α-Phellandrene	1.06	0.02	0.09
α-Fenchene	0.10	0.08	0.17
Linalyl acetate	-	0.05	0.22
Citronellol	0.01	-	0.03
Terpinolene	1.88	2.33	2.41
Linalool	5.09	8.79	6.72
α-Thujone	2.00	1.72	2.60
α-Campholenal	1.36	0.58	0.07
Sabinene	-	0.27	0.44
Cis-sabinene hydrate	-	-	0.30
β-Phellandrene	1.59	3.44	0.62
Cis-thujanol	4.02	5.07	0.88
Trans-4-thujanol	2.01	1.11	0.02
p-Cymen-8-ol	-	1.10	0.21
Trans-calamenene	3.03	-	0.45
Cis-murraola-(4)5-diene	-	-	1.82
Verbenone	-	-	0.52
α-Terpinyl acetate	-	2.03	0.01
Germacrene D-4-ol	-	0.01	2.03
Linalyl acetate	-	-	3.94
Aggregate (%)	75.47	80.77	93.07
Breakdown (%)			
Monoterpenes hydrocarbon (%)	40.50	48.62	51.33
Oxygenated monoterpenes (%)	12.66	14.88	20.49
Sesquiterpenes hydrocarbon (%)	13.79	10.71	14.25
Oxygenated sesquiterpenes (%)	8.83	6.56	7.88

JTLE: Juniperus thurifera leaf extract; TSBE: Juniperus thurifera stem bark extract and JTRE: Juniperus thurifera root extract

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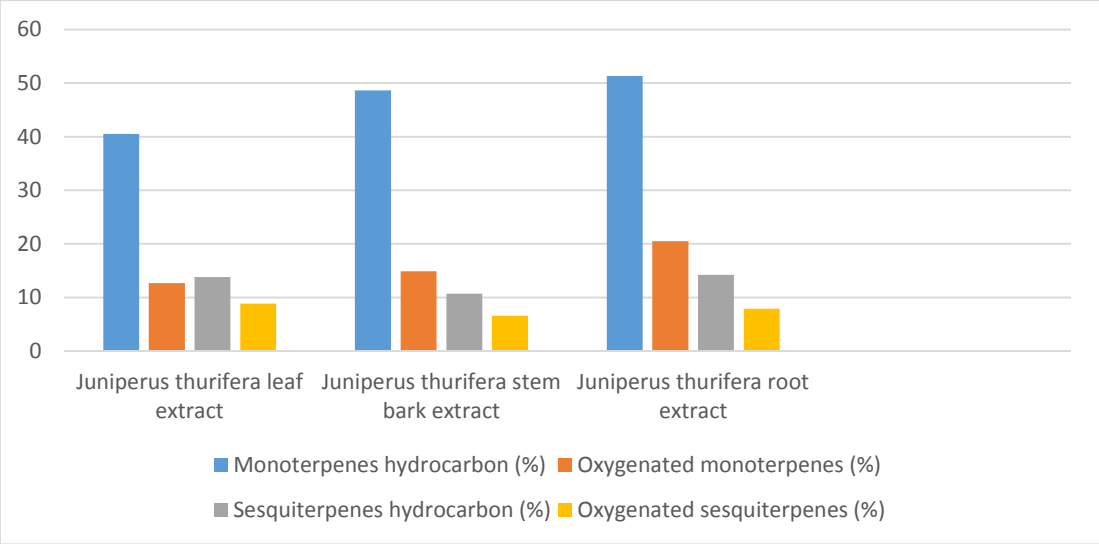

Figure 1: Graphical representation of bioactive compounds in Juniperus leaf, stem bark and root extract

2. RESULTS AND DISCUSSION

Table 1 revealed the gas chromatograph and mass spectrometry (GC-MS) analysis of bioactive compounds in Juniperus thurifera leaf, stem bark and root extract. Result obtained shows that Juniperus thurifera leaf extract contained 33 bioactive compounds (phytochemicals) which accounts to 75.47 % while Juniperus thurifera stem bark and root extract contained 37 and 50 phytochemicals with an aggregate of 80.77 % and 93.07 % respectively. Monoterpenes hydrocarbon (40.50 %), oxygenated monoterpenes (12.66 %), sesquiterpenes hydrocarbon (13.79 %) and oxygenated sesquiterpenes were found in Juniperus thurifera leaf extract while Juniperus thurifera stem bark and root extract contains monoterpenes hydrocarbon (48.72 %, 51.33 %), oxygenated monoterpenes (14.88 %, 20.49 %), sesquiterpenes hydrocarbon (10.71 %, 14.25 %) and oxygenated sesquiterpenes (6.56 %, 7.88 %) respectively. Secondary metabolites in the extracts was highest in Juniperus thurifera root extract, intermediate in Juniperus thurifera stem bark extract and lowest in Juniperus thurifera leaf extract. The result obtained is in agreement with the findings of Barrero et al. (2004); Alagbe et al. (2022) but contrary to the reports of Rachid et al. (2019). According to Oluwafemi et al. (2022); Agubosi et al. (2022) variations in the phytochemical components of medicinal plants could be attributed to species, age of plant, geographical location as well as the extraction procedure employed. Secondary metabolites or phytochemicals are chemicals of plant origin which possess pharmacological actions and are used by plants for defense against competitors, pathogens or predators (Alagbe and Ushie, 2022; Oluwafemi et al., 2022). They can also act as co-factors for enzymatic reactions and substrate for enzymes (Alagbe, 2022; Ramdani et al., 2013), ability to scavenge free radicals capable of causing diseases and selective inhibitors of pathogenic organisms in the body (Hajjouji et al., 2019; Adewale et al., 2021). Terpenes are the largest group of bioactive compounds which may occur as oxygenated derivatives such as ketones, alcohols, aldehydes, phenol oxides and ketones (Muritala et al., 2022; Singh et al., 2021). Flavonoids are group of phenolic compounds with antioxidant, anti-allergic, antiviral, anti-tumor and anti-inflammatory activities (Shittu et al., 2021; Kumar and Pandey, 2013), prevent diseases caused by free

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radicals (Agubosi et al., 2022; Aoki et al., 2000) and prevents cardiovascular diseases (Oluwafemi et al., 2021; Takahashi and Ohnishi, 2004). Phenols are compounds possessing one or more aromatic rings with one or more hydroxyl groups. It has been reported to have antioxidant and anti-inflammatory activities (Olafadehan et al., 2021; Kim and Choi, 2013). Tannins are polyphenolic substance found in many plants product of secondary metabolism. They possess antibacterial, antiseptic and anti-carcinogenic properties (Lopez et al., 2008; Zheng and Wang, 2001), decrease in blood urea nitrogen and inhibition of lipid peroxidation (Amorati et al., 2013; Khan et al., 2009). Alkaloids are alkali-like substances with basic nitrogenous compounds of plant origin possessing a marked physiological function on animals (Hvattum, 2002; Giusti and Wrolstad, 2003).

3. CONCLUSION

It was concluded that Juniperus thurifera leaf, stem bark and root extract contains several bioactive compounds which has marked therapeutic properties. It can also be used as natural alternative to antibiotics to bridge the gap between food safety and livestock production.

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