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Original Article

INVESTIGATION OF CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF ESSENTIAL OILS FROM TWO *SYZYGIUM* SPECIES OF ANDHRA PRADESH, INDIA

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ABSTRACT

Objective: The present investigation is carried out to study the chemical composition, antimicrobial and antioxidant activity of essential oils of two *Syzygium* species i.e. *Syzygium alternifolium* (SA) and *S. samarangense* (SS) leaves.

Methods: The essential oils from *S. alternifolium* (SA) and *S. samarangense* (SS) leaves were obtained by hydro-distillation and analyzed by GC and GC-MS. The oils were subjected to antimicrobial and antioxidant activities by using *in vitro* methods.

Results: Essential oils (EOs) obtained by hydro distillation were analyzed through GC and GC-MS and resulted 25 compounds from each sample. SA leaf oil was dominated by monoterpene hydrocarbons (53.53%) of which, β-mercene (24.04%), β-pinene (9.23%), β-trans-ocimene (9.2%), cyclofenchene (7.21%) and β-cis-ocimene (2.1%). Whereas SS leaf oil was dominated by sesquiterpene components i.e. viridiflorol (15.05%) α-cubebene (7.71%) and monoterpenes, i.e. β-pinene (11.64%), α-pinene (9.61%) and α-terpineol (5.19%). Both essential oils exhibited strong and broad spectrum of antimicrobial activity. The antimicrobial results showed that SA-leaf oil strongly inhibited *Candida rugosa* (CR), *Bacillus subtilis* (BS) and *Staphyloccus aureus* (ST), whereas SS-leaf oil strongly inhibited CR and *Escherichia coli* (EC). Among the test organisms, CR was strongly inhibited by both oils by expression of the lowest minimum inhibitory concentrations (MIC). Further, both the test EOs exhibited concentration dependent DPPH scavenging activity indicating the significant antioxidant property.

Conclusion: *Syzygium alternifolium* and *S. samarangense* leaf essential oils are the good source of natural antimicrobial and antioxidant compounds, which can be used as natural therapeutic agents against human pathogenic organisms.

Keywords: *Syzygium alternifolium, S. samarangense*, Essential oils, GC-MS analysis, Antimicrobial activity, DPPH scavenging activity.

INTRODUCTION

Natural products derived from higher plants may contribute to the search for novel drugs by indicating new modes of pharmacological action. Natural plant products mainly based on the traditional herbal systems are being used in the pharmaceutical industry and primary health care systems in developing countries. In order to find out new sources of drugs, a number of plants have been screened for the wide range of biological activities in various institutions in India. About 3000 materials from 2764 plant species have been screened for their pharmacological and chemotherapeutic properties [1, 2]. But still a vast wealth of medicinal plants have not been explored which contain active medicinal properties to cure diseases. Antimicrobial principles of plant origin have enormous therapeutic potential, which are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [3]. Hence the present study has focused on the two plant species of the genus *Syzygium* with potential medicinal properties and scientifically less explored for the pharmacological studies.

The genus *Syzygium* belongs to the family Myrtaceae, comprising about 1200 species globally and in India, it is represented by 75 species [4]. Globally the genus is distributed in tropical Africa, Asia, Australia, New Caledonia, New Zealand and Pacific Islands [5]. The *Syzygium* species were reported to possess various pharmacological properties viz. antidiabetic, antifungal, anti-inflammatory, antibacterial, antioxidant, antihyperlipidemic and cytotoxic [6].

Syzygium alternifolium (Wt.) Walp. (Myrtaceae) is an endemic aromatic tree species, distributed in Assam and Andhra Pradesh states of India. Locally it is known as mogi/movi (Telugu). The plant parts were used in traditional medicine to cure various diseases viz., tender shoots and fruits for dysentery, seeds for diabetes [7], stem bark was used to treat gastric ulcers [8]. *S. alternifolium* was reported to possess hypoglycemic and antihyperglycemic and antimicrobial activity [8-11] and antioxidant activity [12]. The phytochemical studies revealed only flavonoids and terpenoids isolated from the leaf [13] and the plant material has been unexploited much for detailed studies.

S. samarangense (Blume) Merrill is a deciduous tree, commonly known as samarang apple. It was introduced from Malacca, which is under cultivation in different states of India for their edible fruits. The fruits also used in traditional medicine to cure diabetes. It has been reported to have antidiabetic, antioxidant, analgesic, cytotoxic and anticholinesterase activity [14], α-glucosidase inhibitory activity [15], antiinflammatory [16-19]. The flavonoids isolated from *S. samarangense* were reported to possess antihyperglycemic activity [20], spasmolytic [21] and immunomodulatory activity [22]. Prolyle endopeptidase (PEP) inhibitor chalcones isolated from leaves of *S. samarangense* [23]. Chemical constituents isolated from this plant includes mearnsitrin, 2'-C-methyl-5'-O-galloylmyricitin-3-O-α-L-ramnopyranoside, desmelloxy ma heucinol [24], 4'-6'-dihydroxy-2'-methoxy-3'-5'-di methyl chalcone, methyl 3-epi-betulinate, oleanolic acid, Jacoumaric acid, urosolic acid, arjunolic acid [25], samarngenin A and samarangenin B [26]. Essential oil composition of leaf [27] and flower buds was reported [28].

Thorough review of literature, it was found that, very few reports were noticed on antimicrobial activity of SA leaf and fruit extracts [8-11] and SS leaf essential oil [27]. To the best of our knowledge, there are no previous reports on the chemical composition, antimicrobial and antioxidant activity of SA and SS leaf essential oils. Hence, the present study is designed to evaluate the essential oil

composition, antimicrobial and antioxidant activity of SA-leaf and SS-leaf essential oils by using *in vitro* studies.

MATERIALS AND METHODS

Collection of plant material

S. alternifolum leaves were collected from Tirumala hills and *S. samarangense* leaves were collected form CIMAP-RC, Hyderabad. The voucher specimens were identified by Dr. Venkata Ramana, Osmania University, Hyderabad, with the help of regional and local floras and deposited in Botanical Survey of India (BSI) at Deccan Circle, Hyderabad (Voucher number BSID 000827 and BSID 000832).

Isolation of essential oil

The collected plant material was cut into small pieces and subjected to hydro-distillation for 3-5 h in Clevenger type apparatus and isolated the essential oil [29]. The essential oils were dried over anhydrous sodium sulphate and stored at 4 ºC until used for chemical analysis and biological activities.

Gas chromatography and GC-Mass spectroscopic studies (GC and GC-MS Analysis)

The essential oils from SA and SS leaves were obtained by hydro-distillation were analyzed GC and GC-MS [30]. Essential oil components were identified by comparison of the retention indices of the GC peaks with those obtained using saturated *n*-alkanes (C_8-C_{23}) [31], and confirmation was done with the reported literature [32, 33] as well as NIST-2010 library. Peak area and percentages were calculated from GC-FID response without employing correction factors.

Antimicrobial studies

Test microorganisms

Microorganism used for the present study are *Bacillus subtilis* (BS) (MTCC 1429), *Staphylococcus aureus* (ST) (MTCC 737); *Escherichia coli* (EC) (MTCC 1687), *Pseudomonas aeruginosa* (PA) (MTCC 1688); *Candida albicans* (CA) (MTCC 227) and *Candida rugosa* (CR) (NCIM 3462) were purchased from CSIR-Institute of Microbial Technology (Microbial Type Culture Collection Centre (MTCC)), Chandigarh and CSIR-National Chemical Laboratory {National Collection of Industrial Microbiology (NCIM)}, Pune, India. Bacterial cultures were maintained on nutrient agar (NA) and fungal cultures on potato dextrose agar (PDA) media.

Antimicrobial assay by disc diffusion method

The detailed procedure used for antimicrobial activity of essential oils against test pathogens by disc diffusion method was followed as described in [34].

Determination of minimum inhibitory concentration (MIC) of essential oils

The minimum inhibitory concentration (MIC) was assayed by broth microdilution method with slight modifications by using 96-well micro titer plate [34].

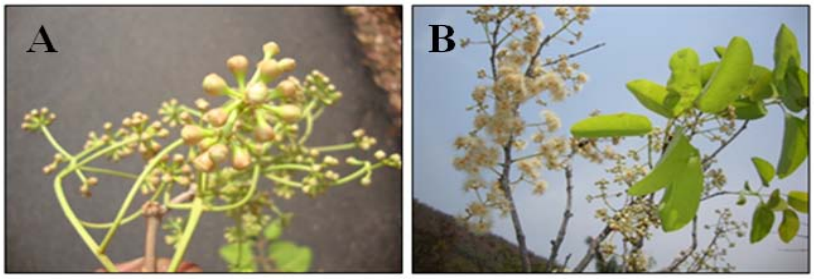

Fig. 1: *Syzygium alternifolium* (A) and *S. samarangense* (B) flowering condition

Antioxidant activity

Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The quenching effect of SA and SS leaf essential oil samples was assayed using the stable free radical, 2,2-diphenylpicrylhydrazyl (DPPH), as a reagent by spectroscopic method [34]. The percent (%) DPPH reducing capacity of oil samples were calculated by using the following formula:

$$\% = [(Ac-As)/Ac] \times 100$$

Where Ac is the absorbance of the control reaction (containing all reagents except the test sample/standard), and As is the absorbance of the test sample. Ascorbic acid was used as the standard compound. The concentration of the oil sample required for 50% inhibition (IC_{50}) was calculated form the standard graph plotted of inhibition percentage against extract concentration. Tests were carried out in triplicate and mean values were tabulated along with the standard error.

Statistical analysis

Studies were performed in triplicate, and the mean value was calculated. The means were analyzed by two-way analysis of variance (ANOVA) used Window stat 8.5 advanced level statistical software. The results were expressed critical difference (CD) at 5% were considered as significant.

RESULTS

Essential oil composition

The hydro distillation of *S. alternifolium* fresh leaves gave pale yellow in color essential oil with fruity smell (0.27 %). The essential oil sample was analyzed using Gas Chromatography coupled with Mass spectroscopic method. The identified compounds were tabulated according to their relative percentage and relative retention indices (table 1). Twenty five compounds were identified which constituted 96.6 % of the total oil.

The oil was characterized by a high concentration of monoterpene hydrocarbons (53.53%) of which, β-myrcene (24.04%), β-pinene (9.23%), β-trans-ocimene (9.2%), cyclofenchene (7.21%) and β-cis-ocimene (2.1%). Oxygenated monoterpenes (5.0%), sesqui terpenoids comprised (34.55%), oxygenated sesquiterpenoids (5.81%), and oxygenated hydrocarbons (0.41%) and aromatic hydrocarbons (0.7%) constituted of the total oil.

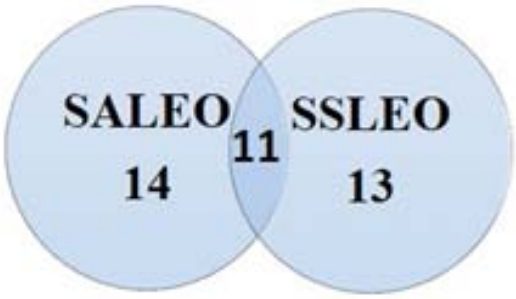

Fig. 2: Ven diagram showing distribution of essential oil components in SA and SS leaf essential oils

Table 1: Chemical composition of the leaf essential oil from *Syzygium alternifolium* (Wt.) Walp and Syzygium *samarangens* (Blume) Merrill & Perry

Compound Name	Retention Index	Area of %	
		SA	SS
Cyclofenchene	729	7.21	-
6-Methyle-5-hepten-2one	938	0.34	-
α-Pinene	948	-	9.61
β-Pinene	943	9.23	11.64
β-Myrcene	958	24.04	1.44
β-Trans-Ocimene	976	9.2	2.81
β-Cis-Ocimene	976	2.1	2.2
D-Limonene	1018	1.75	1.19
Linalool	1082	2.47	2.19
1-Terpinen-4-ol	1137	0.4	-
α-terpineol	1143	1.48	5.19
p-Cymene-8-ol	1197	0.46	-
Copaene	1221	-	1.36
Nerol acetate	1352	0.31	-
1-H-Cycloprop (e) azulene	1386	-	1.55
Guaia-1(10),11-diene	1419	-	1.28
Norbornane	1425	0.2	-
γ-Murrolene	1435	-	3.45
α-Amarphene	1440	-	3.25
β-Farnesene	1440	17.06	-
Napthalene	1440	-	1.86
(-)Zingiberene	1451	1.42	-
Farnesene	1458	4.84	-
α-Farnesene	1458	0.34	-
α-Humulene	1461	0.75	2.08
δ-Cadenene	1469	-	7.71
γ-Selinene	1481	-	1.16
Caryophyllene	1494	9.94	1.34
Caryophellene oxide	1507	1.94	1.52
Curcumene	1524	0.24	-
Ledol	1530	0.47	3.48
Viridiflorol	1530	-	15.05
Neroledol	1564	0.44	-
α-Caryophyllene	1579	-	1.86
1-Naphthalenol	1621	-	11.07
α-Bisabolol	1625	2.96	-
Hedycaryol	1694	-	3.57
Bromo-8-tetrahydropyranyleoxyoctane	1837	0.41	-
Aromatic Hydrocarbon		0.70	12.93
Oxygenated Hydeocarbons		0.41	
Monoterpene Hydrocarbons		53.53	30.27
Oxiginated Monoterpene Hydrocarbons		5.00	7.38
Sesquiterpene Hydrocarbons		34.55	20.04
Oxiginated Sesquiterpene Hydrocarbons		5.81	23.62
	Total	100.00	99.24

S. samarangense leaf essential oil was dominated by sesquiterpenes (48.66%), followed by monoterpenes (37.65%) and aromatic compounds (12.93%). The major sesquiterpene components are viridiflorol (15.05%) α-cubebene (7.71%), followed by monoterpenes, i.e. β-pinene (11.64%), α-pinene (9.61%), α-terpineol (5.19%) and 1-naphthalenol (11.07%) from aromatic hydrocarbons. Eleven compounds i.e. β-Pinene, β-Myrcene, β-Trans-Ocimene, β-Cis-Ocimene, D-Limonene, Linalool, α-Terpineol, α-Humulene, Caryophyllene, Caryophellene oxide and Ledol were common compounds in both oils (fig. 2).

Antimicrobial studies

The antibacterial and anti candidal activities of the SA-leaf and SS-leaf oil were determined by disc diffusion method. Minimum inhibitory concentration (MIC) of the both oils was assayed by broth microdilution method using 96 well micro titter plates.

The results showed that essential oil of SA-leaf strongly inhibited CR, BS and ST, whereas SS-leaf oil strongly inhibited CR and EC (Fig.3). Of the test organisms, CR was strongly inhibited by both oils by expression of the lowest MIC values (fig. 4).

Antioxidant studies

The antioxidant capacity of both essential oils and standard compound ascorbic acid was assayed by using DPPH reagent. Both the test oils strongly reduced DPPH purple color in the concentration dependent manner (fig. 5). This activity is similar to that standard compound.

DISCUSSION

Essential oils (EOs) are volatile aromatic oily liquids obtained from different parts of medicinal and aromatic plants. The main constituents of the EOs are mono and sesqui terpenes, which are responsible for the aroma, flavor and fragrance associated with aromatic medicinal herbs [35]. Previous scientific reports suggested that, plant derived EOs are considered as therapeutically effective against several human pathogenic microorganisms. Besides antimicrobial properties, EOs reported for antiviral, antimutagenic, antiparasitic and insecticidal properties [36].

Therefore, they can be used as herbal medicine as natural antibiotics to control human pathogenic bacterial and fungal species. Natural

antioxidants have many industrial uses, such as preservatives in food and to prevent the degradation of rubber and gasoline [37]. The benefits of antioxidants are very important to maintain good health, because of its free radical scavenging capacity. The human body naturally produces free radicals and antioxidants to prevent their damaging effects.

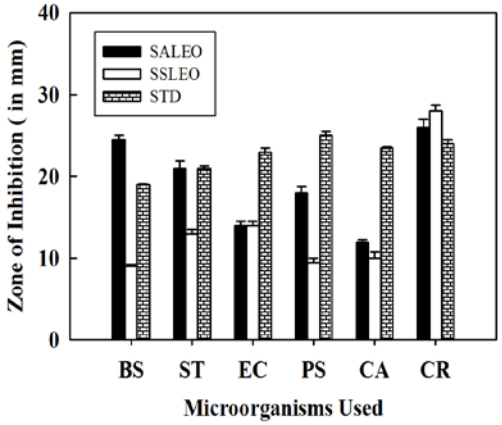

Fig. 3: Antimicrobial activity of *S. alternifolium* and *S. samarangense* leaf EOs against the test pathogens. The assay was performed by agar disc diffusion method

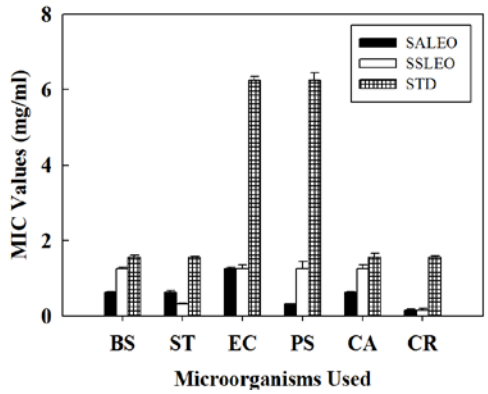

Fig. 4: Minimum Inhibitory Concentration (MIC) values of the *S. alternifolium* and *S. samarangense* leaf EOs against the test pathogens. MICs of the test oil samples were determined by broth micro dilution method

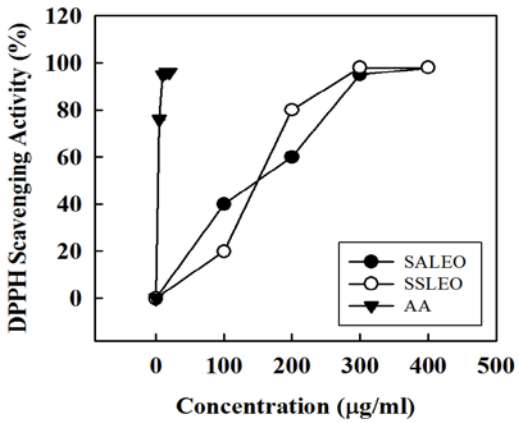

Fig. 5: DPPH scavenging activity of *S. alternifolium* and *S. samarangense* leaf EOs

Note: The error bars are very less and the inhibition was significant ($p<0.05$).

However, in most cases, free radicals far outnumber the naturally occurring antioxidants. In order to maintain the balance, a continual supply of external sources of antioxidants is necessary in order to obtain the maximum benefits of antioxidants. Therefore, natural antioxidants helps the body by neutralizing, removing the free radicals from the blood stream, protects the cells or tissues against their toxic effects and contribute to disease prevention [38].

The present study was focused on the chemical composition, antimicrobial and antioxidant properties of EOs of two *Syzygium* species i.e. *S. alternifolium* and *S. samarangense* leaves. Chemical composition of the essential oils was determined by GC-MS analysis Table1. *S. alternifolium* leaf oil strongly inhibited CR and BS, which was dominated by β-myrcene, β-farnesene, caryophyllene, β-pinene, β-t-ocimene, cyclofenchene, α-bisabolol, linalool, may be responsible for potent antimicrobial activity. β-myrcene is known for its biological activity. α-and β-pinene and limonene have been reported for its antibacterial activity [39-40]. α-and β-pinene are able to destroy cellular integrity, and inhibit respiration and ion transport processes [41-42]. Broad spectrum of antimicrobial activities of essential oils was might be due to their complexity and variability of chemical constituents. To the best of our knowledge, this is the first report on the chemical composition, anticandidal and antioxidant activity of *S. alternifolium* leaf essential oil.

S. samarangense leaf oil exhibited strong inhibition against CR and EC. It was dominated by sesquiterpene compounds (30%). Sesquiterpene compound viridiflorol, which is rich in SS leaf oil was reported to possess antifungal and antibacterial activities [43-44]. Caryophyllene and caryophyllene oxide, which is found in SS leaf oil in minor quantity, has been reported for pharmacological properties like, antibacterial [45], antifungal [46], antiplatelet aggregation [47] and cytotoxic activities [48]. Monoterpene, Terpenen-4-ol is one of the minor compound of the both EOs was contributed to be responsible for bacteriostatic activity against several microorganisms [49]. Linalool is a dominant constituent of a number of essential oils, was reported for antimicrobial [50], antinociception [51], antileukemic [52] and antispasmodic activity [53]. Toxicological studies has been reported that linalool is relatively safe as a topical or inhalation agent [54]. Antimicrobial activity of *S. samarangense* leaf essential oil was reported by Joji Reddy and Bena Jose [27]. The present study highlights the anticandidal activity of *S. samarangense* leaf essential oil and the more number of chemical compounds than the previous report [27].

Both essential oil contained high levels of monoterpenes and sesquiterpenes showing a highly antioxidant activity. It was shown that the terpene hydrocarbons, whose antioxidant activity is similar to that of phenolic compounds, which break the free-radical chain reactions, which could be accompanied by their irreversible oxidation into inert compounds [55, 56]. In addition, the essential oils with monoterpene hydrocarbons and sesquiterpenes possess greater antioxidant properties [57]. In the present investigation, the tested essential oils exhibited a concentration-dependent antiradical activity by scavenging the DPPH–radical. Both the essential oils with the concentration of 300 µg/ml exhibited significant free radical scavenging activities (>85 %) and it is concentration dependent. The scavenging effect of Ascorbic acid (standard compound) on DPPH showed higher than that of the extracts at each concentration points.

CONCLUSION

Nowadays, the increasing demand for natural drugs for better health and safety products and also free for chemical additives, antibiotic and synthetic drugs resistant microorganisms have led to a considerable increase of the use of EOs. The increased attention in alternative natural substances is motivating the research community to find new habits and applications of these EOs. In fact, they are used directly into the food matrix as food additives or encapsulated in coating and edible films to minimize the organoleptic effect of EOs. In addition, the EOs are employed as nutrient substituent for improvement of animal health and for topical use which prevents the internal as well as external parasites. Hence the antioxidant and antimicrobial activities of selected two *Syzygium* plant species might be responsible for their therapeutic properties which provide

scientific support for the usage in various human ailments in the traditional system.

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CONFLICT OF INTERESTS

Declared None

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