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SECONDARY METABOLITES AND THEIR BIOACTIVE POTENTIAL ANALYZED BY GC-MS IN *STROBILANTHES ALTERNATA* (BURM.F.) MOYLAN EX J.R.I.WOOD

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Abstract:

Bioactive secondary metabolites in the leaf extracts of Strobilanthes alternata were analysed using GCMS. The alcoholic extract of the leaves contain eight fatty acid methyl esters, five terpenoids and aromatic dicarboxylic acids distributed in varying concentrations. Squalene which occurred very low quantity compared to other molecules is reported to possess antioxidant potential and contribute considerable to the wound healing property. Most of the molecules are known to possess antibacterial, antifungal and anti-inflammatory activities. Many of the biomolecules are characterized by antioxidant potential also. The process of wound healing in which S.alternata known to play a vital role which is discussed in terms of the combined activity of each and every molecules of the leaf extracts possessing antimicrobial, anti-inflammatory and antioxidant activities. Wound healing and antioxidant potential of bioactive molecules are seemed to coexist in S.alternata.

Key Words: Strobilanthes alternate, GCMS, Antioxidant, wound healing, Antimicrobial

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1. INTRODUCTION:

More than four billion population of the world, that is about 80% of the world population presently use the traditional system of medicines or herbal drugs for primary health care. In India more than 65% of the total population use herbal medicinal products for the treatment of diseases [1].

For centuries, plants have been used to treat diseases worldwide and still performing a major role in health care system in many developing countries. The use of plant extracts and plant derived components in the treatment and management of wounds have been well observed [2,3,4,5].

Analysis of bioactive components of green plants leaves using GC-MS studies have been conducted in many medicinal plants. Important aspect repeatedly reported by these investigation is the phytochemical activities or potential of the extracts aqueous/organic solvents on microbes or on test animals . GC-MS analysis of *Baccopa monerrii* revelaed qualitative and quantitative distribution of saponins[6] . Singh and Aeri ,(2013) analyzed secondary metabolites of *Acanthes ilicifolius* and reported components like saponins, tannins, alkaloids, flavonoids and triterpenoids[7]. *Crotton bonplanbianum* plants revealed the occurrence of many secondary components possessing antibacterial, antitubercular and anticytotoxic activity by GC-MS studies[8]. According to Abid and Touquer (2015) phytochemicals of *Breynia disticha* include antimicrobial and antioxidant activities in methanolic extracts[9]. Tamboli *et al*.,(2018) compared the presence of phytoconstituents like alkaloids, phenolics, saponins and pyrenoids in natural and micropropagated from *Bacopa* plants[10].

Almost all studies using GC-MS to analyse bioactive components are centred around the identification and estimation of these metabolites of plants in general and medicinal plants in particular. GC- MS analyses of phytochemicals and potentially bioactive components of many medicinal plants have been reported in plants like *Euphorbia kansuii* [11], *Cassia italic*[12] , *Artimesia princeps* [13], *Moringa peregram* [14], *Vernonia elangifolia* and *Bregaria disticha* [9], *Moringa oliffera* [15], *Huru crepitans* [16], *Ipomea violaeae* [17], Equisetum arvanse [18], *Bacopa monnerri* [10], *Homolomena aromatic* [19].

Antibacterial, antifungal, antioxidant and/or anti-inflammatory activities of several medicinal plants inclusive of *S.alternata* have been elaborately studied and reported. Excision wound healing and anti-inflammatory properties of *Hemigraphis colorata* leaves were reported earlier [20,21,22]. Antibacterial activity of the plant was investigated against selected pathogens [23]. Antimicrobial, anti-inflammatory and antioxidant properties of *H.colorata* leaf extract was also reported [24]. Nevertheless, characteristic potential and activities of each component of the extracts of *S.alternata* have not yet been studied in detail.

In the present study, the qualitative and functional role of individual bioactive component separated by GC-MS in the methanolic extract of *S.alternata* (Syn: *Hemigraphis colorata*) leaves are compared and discussed in relation to the specific phytochemical characters or activities of each component molecules. Comparison is also made with the reported/attributed medicinal properties of the plant extracts irrespective of the solvent system used. *Strobilanthes alternate* (Burm.f.) E. Moylan ex J.R.I. wood (syn: *Hemigraphis colorata*) belongs to the family Acanthaceae. In kerala, the plant is popular in the name “Murikootti” and “Murianpacha”, due to its incredible potency to heal fresh wounds [20]. It is prostrate plant spreads with rooting stem. Leaves are entire, lences shaped, toothed or lobed margin. Leaves of *S.alternata* are used for wound- healing purposes.

Keeping in view the antimicrobial and anti-inflammatory properties and other medicinal uses reported earlier, *S.alternata* is to be analyzed by GC-MS to evaluate the function of each and every bioactive components. Hence the present study was undertaken to separate and identify the secondary metabolites by GC- MS method to assess the function or vital role of the constituents of the leaf extract. The significance of leaf components in the processes of wound healing partaning to the antimicrobial and anti-inflammatory activities with more emphasis on antioxidant potential of specific bioactive molecules of *S.alternata* is the prime objective of this investigation.

2. MATERIALS AND METHODS:

2.1 Choice of plant

Mature leaves of healthy twigs of *Strobilanthes alternate* plants growing in the botanical garden, SNGS College Pattambi were collected and used for the study.

2.2 Preparation of leaf extract

Fresh leaves were collected and shade dried. Dried leaves were grounded into fine powder and 5g of the powder was subjected to extraction using 100ml of

methanol in soxhlet apparatus. After running several cycles, the extracts obtained was concentrated and was used for GC-MS analysis.

2.3 GC-MS Analysis

Gas Chromatography Mass Spectrometry analysis of leaf extracts was performed using Shimadzu GC-MS, with model number QP2010s, at Kerala Forest Research Institute, Thrissur, Kerala. Helium gas was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 1 µL was employed. The injection port temperature was set at 260ºC and ion source temperature at 200ºC. The oven temperature was programmed from 80ºC for 4 minutes with an increase of 5ºC/min to 280ºC with a hold time of 6 minutes. The components were identified based on the comparison of their relative retention time and mass spectra with those of wiley NIST7Nlibrary data.

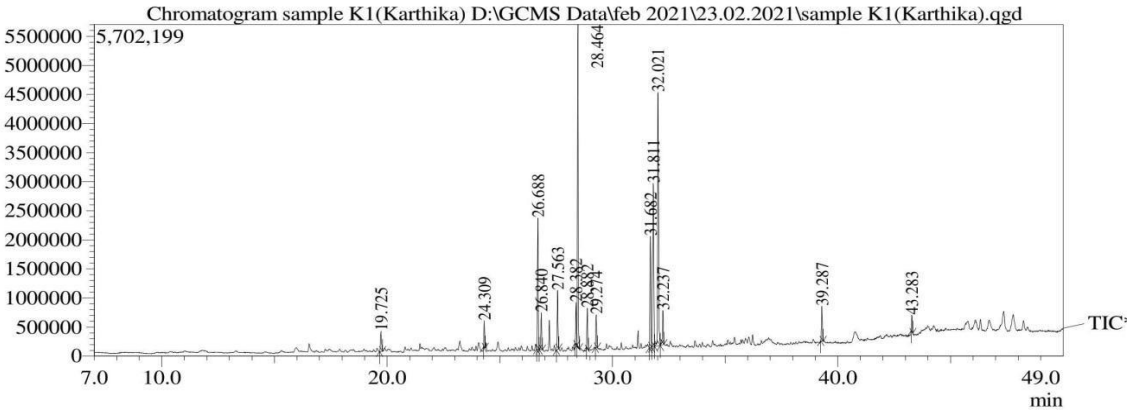
3. RESULTS AND DISCUSSION:

GC-MS analysis of methanolic extract of *S.alternata* resulted in the identification of 15 secondary metabolities. Those compounds identified are Neophytadine, 3,7,11,15-Tetramethyl 2 hexadacen-1ol, Methyl palmitate, Methyl octadeca 9,12 Dienoate, Methyl laurate, Methyl myristate, Hexahydrofarnesyl acetone, 9-octadecenoic acid, Isophytol, 2,5 Dimethyl-4 Hexen 3 ol, Linolenic acid, Phytol, Methyl stearate, 1,2 Benzene dicarboxylic acid and Squalene. Quantitative expression of these secondary metabolities was expressed by considering the area percentage of GC-MS profile(Table-1:Chromatogram- 1).

TABLE-1 GCMS DATA

SI.NO	NAME OF THE SECONDARY METABOLITES	AREA PERCENTAGE
1	Neophytadine	9.09
2	Hexahydrofarnesyl acetone	2.68
3	Isophytol	2.60
4	Phytol	20.57
5	Squalene	1.02
6	3,7,11,15 tetramethyl 2 hexadecen 1 ol	4.07
7	Methyl palmitate	22.28
8	Methyl laurate	1.80
9	Methyl myristate	1.55
10	9-Octadecenoic acid	2.69
11	2,5 dimethyl 4 hexen 3 ol	2.31
12	Linolenic acid	16.05
13	Methyl stearate	2.77
14	1,2-benzene dicarboxylic acid	2.66
15	Methyl octadeca 9,12 dienoate	7.86

CHROMATOGRAM OF GCMS DATA



Considering the area percentage abundantly occurring component is Methyl palmitate(22%). Second abundantly occurring compound was phytol (20%) followed by Linolenic acid(16%).In addition to the above components the following fatty acid methyl esters were also present as given below.
Methyl octadeca 9,12 dienoate (7.86%), 3,7,11,15 Tetramethyl 2 hexadecen 1-ol (4.07%), Methyl stearate (2.77%), 9-Octadecenoic acid(2.69%), Methyl laurate (1.80%) and Methyl myristate (1.55%).Other than the Fatty acid methyl esters, six terpenoid compounds also were identified in the GC-MS analysis of *S.alternata* leaves. Phytol with area percentage 21% is the abundantly occurring monounsaturated diterpene alcohol. Neophytadine was second abundantly occurring terpenoid (9%) belonging to sesquiterpenoid. Hexahydrofarnesyl acetone content was 2.7% and Isophytol was 2.60%. Least distributed bioactive compound was squalene, a triterpene present only 1.02%.1,2-Benzene dicarboxylic acid(2.66%) was an aromatic dicarboxylic acid present in *S.alternata* other than the terpenoids and fatty acid methyl esters.

Medicinal property of *H.colorata* is known earlier and used for wound healing as a folk medicine. Research publication on medicinal use of this plant reveals the antimicrobial [23] and anti-inflammatory properties[20]. Antioxidant activity has also been mentioned recently[24],[25] . All these investigations are based on experiments with microbes and for test animals subjected to treatment with aqueous and alcoholic extracts of *S.alternata*. However the principles and/or phytochemical roles of the component of the extracts have not yet been delineated.

GCMS study on alcohol extract of *S.alternata* (*H.colorata*) revealed the occurrence of a number of bioactive components (Table- 1) of which many molecules possess active principles pertaining to the medicinal use for wound healing. Methyl palmitate is abundantly occurring in *S.alternata* and this component is having anti-inflammatory property [26,27]. Antiinflammatory potential of this plant is found to be due to the presence of Neophytadine, 3,7,11,15-Tetramethyl 2 hexadecen 1 ol because these molecules are known to play anti- inflammatory role in accordance with the view[28]. Another bioactive molecule present in *S.alternata* is phytol which is the second abundant component possessing antimicrobial, antinociceptive, antioxidant and immunostimulant activities as opined by Rhy *et al*.,(2011) and Santos *et al*.,(2012)[29,30]. Hexahydrofarnesyl acetone (sesquiterpene), Isophytol are potential molecules characterized by antibacterial and antifungal properties as reported by VenkataRaman *et al*.,(2012). Methyl stearate is present in the leaf extract of *S.alternata* [28]. Ukwubile *et al*.,(2019) stated that methyl stearate isolated from *Melastromatum capitatum* play a vital role in health care systems and according to those authors presence of methyl stearate and fatty acids in the leaves of the plant might be responsible for its biological activity in traditional medicine[31].

Antifungal activity of fatty acid methy esters of vegetable oils has been demonstrated and evaluated in case studies on fungus [32]. Antioxidant potential was demonstrated by scavenging effect of these fatty acid methyl esters on 2,2-diphenyl 1- picryl hydrazyl (DPPH) radicals.
Antioxidant activities of many secondary metabolite molecules is well known and excellently interpreted in the process of wound healing [33,34]. 3,7,11,15-tetramethyl 2 hexadacen 1 ol, 9-octadecenoic acid, phytol, fatty acid methyl esters and squalene are the bioactive components present in *S.alternata* which

are characterized by antioxidant potential[28,30,32,36] . Antioxidant potential of *S.alternata* extract has been demonstrated by DPPH assay[24,25].

Houghton *et al*.,(2005) reported the involvement of antioxidant components in wound healing process has in *Secamone alzeli* and *Spathodea campanulata*)[35]. In addition a number of plants that have been reported to show antioxidant potential coming under different families[34]. Squalene is a triterpene which occurs comparatively in very small quantity in *S.alternata* (Table-1). According to Lozano-Granta *et al*.,(2018) squalene is a biochemical intermediate possessing antioxidant activity present in all vegetable oils[37]. Aioi *et al*.,(1995) reported the scavenging role of squalene on ROS produced by oxidative stressors and have a protective role of this molecule in combination with SOD[38]. Squalene is abundant in skin which is the target tissue exposed to environmental stresses leading to oxidative stress and hence squalene plays an antioxidant role in protecting the skin due to the specific role of squalene [36].

Shetty *et al*.,(2007) interpreted that wound healing is the process of repair that follows injury to the skin and initial stages of wound healing involves an acute inflammatory phase[33]. According to those authors, the antioxidant properties of *Ocimum sanctum* is found to be responsible for faster wound healing in rats. Incision wounds in rats resulted in the increased antioxidant enzymes and *Ocimum* extract treatment showed decreased activity indirectly establishing antioxidant potential of *Ocimum sanctum* in wound healing[33] .

According to Dissemond *et al*.,(2002) in the therapy of chronic wounds
overproduction of ROS results in the oxidative stress, thereby causing cytotoxicity and delayed wound healing[39]. Antioxidants like SOD, catalase, Glutathione etc hasten the process of wound healing by destroying the ROS .It is known that the wound healing process can be aided by the presence of antioxidants and 36 plants have been reported to possess wound healing and antioxidant activity[34].

As mentioned earlier, besides antibacterial and antifungal properties, antioxidant potential is another important property of squalene[37]. According to those authors, in human body squalene is secreted by sebaceous glands for skin protection and forms 10-15% of lipid on skin surface and on internal organs such as liver. This behaviour of squalene is found to be correlated with the practise of topical application of leaf juice of *S.alternata* to fresh wounds for immediate healing is consertent with the view of Subramaniam *et al*.,(2001)[20]. Antioxidant property of squalene is also related to the protection by scavenging the ROS produced due to wound- induced stress whereas antimicrobial activity of those components of the leaf juice performs an aseptic condition to enhance wound-healing indicating an antiseptic property of *S.alternata* resulting in immediate healing of wounds.

The antioxidant properties of squalene and use as a topical skin lubricant and protectant can also be correlated with wound healing in consonance with the view of Micera *et al*.,(2020) according to whom squalene possess antioxidant activity and is abundant in skin which is the target tissue exposed to wounds or other incisions[36].

The vital role of antioxidants in the process of wound healing is found to be due to the presence of phytol, squalene, 9-octadecenoic acid, 3,7,11,15 tetramethyl 2 hexadacen 1 ol, methyl palmitate and other fatty acid methyl esters of *S.alternata*. This observation is in accordance with the view of Suntar *et al*.,(2007) according to whome wound healing and antioxidant activity coexist in many plant species of a variety of families[34]. As mentioned earlier, wound healing capacity of *S.alternata* is already known and presumed to involves antimicrobial, anti-inflammatory and antioxidant activities of leaf juice[20]. The phytochemical functions of each bioactive component of leaf extract play a vital role in the process of wound healing which is found to be a conserted activity of molecules present in the leaf extracts. Supplementary role of hexahydrofarnesyl acetone, methyl stearate, linolenic acid and 9-octadecenoic acid compounds in the process of wound healing and its complementary role cannot be ruled out.

4. CONCLUSION:

Bioactive property of secondary metabolites in leaf extract of *Strobilanthes alternata* reveals the antimicrobial, anti-inflammatory and antioxidant activites. The involvement of these molecules in the process of wound healing is a known character of the plant is found to be due to the conserted activity of 15 biomolecules many of them possess antioxidant activity and hence wound healing and antioxidant activity coexist in *S.alternata*.

5. DECLARATION OF COMPETING INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and

writingof this article.

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