

Antimicrobial and Anti – inflammatory activities of *Plectranthus amboinicus* Leaf extract on Silkworm Pathogen *Streptococcus pyogenes* SA 1

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ABSTRACT: To study the antimicrobial effect of herbal plant *Plectranthus amboinicus* on the silkworm pathogen *Streptococcus pyogenes* SA1. The herbal plant *P. amboinicus* - leaf was extracted by using known solvents such as acetone, methanol and distilled water and the antimicrobial effect of these extracts were studied against *S. pyogenes* SA 1 by agar well diffusion method. The phytochemical constituents of these extracts were also determined and GC-MS analysis was done. The phytochemical analysis of acetonic extract of *P. amboinicus* - leaf was revealed the presence of alkaloids, flavonoids, coumarins, steroids, glycosides, carbohydrates, tannins and saponins. The acetone and the methanol extracts of *P. amboinicus* - leaf showed minimum inhibitory activity 10 ± 0.03 mm and 3 ± 0.08 mm respectively in 0.5µl concentration and maximum inhibitory activity 18 ± 0.15 mm, 10 ± 0.01 mm respectively against bacterial pathogen *S. Pyogenes* SA 1. The distilled water extract of *P. amboinicus* - leaf showed no inhibitory activity and maximum inhibitory activity 2 ± 0.004 mm in 2.0 µl concentration on *S. Pyogenes* SA 1. The GC-MS analysis of the acetone extract of *P.amboinicus* – leaf was showed the presence of 18 compounds. The acetone and methanol extract of *P. amboinicus* – leaf showed remarkable antimicrobial activity against silkworm pathogen *S. pyogenes* SA 1 and the GC-MS analysis of the acetonic extract of *P. amboinicus* – leaf showed the presence of various antimicrobial compounds. In vitro and in vivo tests have to be carried out to confirm these findings.

Keywords: *Plectranthus amboinicus*, chemical composition, GC-MS, antimicrobial activity.

INTRODUCTION

Herbal plants are the local heritage with global importance and it is blessed with diversity of herbal plants (Suriyavathana *et al.*, 2010). In developing countries, these plants are used by 80 % of the world population and it is remedy for various diseases (Hashim *et al.*, 2010). The herbal plants are the economically important plants that provide the ingredients for manufacture various pharmacologically important medicinal products (Nataraj and Ramachandramurty 2014). The vast majority of plant extracts showed certain biological characteristics that are used in medical treatments (Bhavadhaniparkavi and Abirami 2023). Both traditional and contemporary healthcare systems continue to depend on the therapeutic benefits of medicinal plants (Sheeba *et al.*, 2023).

The genus of the plant *Plectranthus* consists of 300 species, distributed from Africa to Asia and Australia (Waldia *et al.*, 2011). In India, there are about 30 *Plectranthus* species are known and among these

species *P. amboinicus*, *P. vettiveroides*, *P. barbatus*, *P. mollis*, *P. coetsa*, and *P. incanus* are the most common species used in the traditional Indian ayurvedic medicines (Sripathi and Ravi 2017). In India *Plectranthus amboinicus* grows naturally and is distributed on the plains of Rajasthan and Uttarakhand districts of the Western Ghats of India (Lukhoba *et al.*, 2006). Due to the presence of numerous significant constituents or secondary metabolites like flavonoids, glycosides, phenols, tannins. The plant has medicinal properties such as antimicrobial activity, antifungal activity, anti-inflammatory activity, anti-inflammatory activity, antidiabetic activity, anxiolytic activity, antineoplastic, analgesic, antimalarial, antibiofilm efficacy, diuretic, wound healing activity, skincare, respiratory disorders (Punet Kumar & Kumar 2020). This plant is used by traditional healers to treat cough, colds, stomach flatulence, indigestion, constipation, and other conditions (Sahu *et al.*, 2022). The species *P. amboinicus* has a scientific synonym known as *Coleus amboinicus* which is routinely used in culinary services, and as a phototherapeutic product which is also used as

a primary raw material in the pharmaceutical industry (Souza and Lorenzi 2005). Its constituents identified by chemical and phytochemical assays, include germacrene, thymol and carvacrol have excellent antimicrobial and anti-inflammatory activities (Bandeira *et al.*, 2011). Some of the pharmacological properties that have been recognized for *P. amboinicus* include anti-epileptic, urolithiasis, anti-tumor, antimutagenic radio protective, anti-microbial, neuro pharmacological and diuretic properties (Patel *et al.*, 2010). Herbal plants are composed of complex chemical substances of different proportion which occur as secondary metabolites. The phytochemicals are classified into two groups, namely primary and secondary constituents as per their specific functions in plant metabolism. The primary constituents comprise common sugars, amino acids, proteins and chlorophyll, while secondary constituents consist of alkaloids, terpenoids, flavonoids, tannins and phenolic compounds (Krishnaiah *et al.*, 2007).

At present, the interest for the study of the organic chemical compounds from the herbal medicinal plants and their important activity has increased. A lot of extraction methods and analytical methods such as spectrophotometry, capillary electrophoresis, Gas Chromatography (GC) with Flame Ionization Detection (FID), Gas Chromatography - Mass Spectrometry (GC-MS) are developed for identification of plant active compounds. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC-MS an ideal technique for determination of qualitative and quantitative volatile and semi volatile compounds (Iordache *et al.*, 2009). In the view of the medicinal values of the selected plant *P. amboinicus*, the present study is aimed to screen the antimicrobial bioactive compounds and their effect on the silkworm pathogen *S. pyogenes* SA 1.

MATERIALS AND METHODS

Collection and preparation of herbal plant material.

The leaves of herbal plant *P. amboinicus* were collected from Vilavancode taluk, Kanyakumari District, Tamilnadu, India. The plant leaves were taken to the laboratory and air dried at room temperature and crushed into powder for extraction.

Isolation of bacterial pathogen. The bacterial pathogen *S. pyogenes* SA 1 was isolated from the disease infected mature fifth stage silkworm larva. The isolated pathogen was identified through morphological, biochemical and 16S rRNA technique (Accession no: OL452034).

Preparation of herbal plant extract. The herbal plant *P. amboinicus* leaf powder each 10 gram with triplicates were taken in 250 ml conical flask and individually soaked in 100 ml of solvents such as the acetone, methanol and distilled water. Then, the mixture was kept on the shaker and kept undisturbed for 24 hours. The above preparation was filtered with Whatman No. 1 filter paper and the filtrate was concentrated under room temperature to get a semi solid extract (Bag *et al.*, 2009).

Determination of antimicrobial activity.

Antimicrobial activity of different plant extracts was determined by the agar well diffusion method. The bacterial strain *S. pyogenes* SA 1 was swabbed on the solidified nutrient agar media, then 4 wells with 6 mm diameter were punched in the agar media by using sterile metallic borer. Stock solutions of crude extract and fractions in dimethyl sulfoxide (DMSO) at concentration of 20 mg / ml was prepared and 0.5, 1.0, 1.5 and 2.0 μ l of stock solution were added into the respective wells (Choudhary and Thomsen 2001). The petri dishes were incubated at 37°C for 24 hours. Then, the plates were observed for zone of inhibition. The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition against the tested bacterial pathogen (Jain *et al.*, 2009).

Phytochemical analysis. Phytochemical analysis of the crude extract of acetone, methanol and distilled water fraction of *P. amboinicus* was carried out by using the standard procedure. Crude extracts were subjected to the initial phytochemical analysis for the presence of alkaloids, flavonoids, coumarins, steroids, glycosides, tannins, carbohydrates and saponins (Gnanavel *et al.*, 2015).

Gas chromatography - mass spectroscopy (GC-MS)

analysis. The analysis of the acetone fraction of *P. amboinicus* - leaf was carried out by using Perkin Elmer workstation, with model Clarus 680 GC which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer instrument employing the following condition: Elite – 5MS capillary column (30.0m \times 250 μ m, film thickness 0.25/m) and the components were separated using Helium as carrier gas at a constant flow of 1ml/min. The injector and MS transfer line temperatures were set at 260°C during the chromatographic run. The 1 μ l of extracted sample injected into the instrument, the oven temperature was as follows: 60°C (2min); followed by 300°C, where it was held for 6min. The mass detector conditions were: transfer line temperature 230°C; ion source temperature 230°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GCMS, National Institute of Standards and Technology (NIST) library (Adams, 2001).

RESULTS AND DISCUSSION

Determination of antimicrobial activity. The *P. amboinicus* leaf extracts were showed antibacterial activity against the selected silkworm pathogen *S. pyogenes* SA 1. The results revealed that the acetone extract of *P. amboinicus* – leaf on *S. pyogenes* SA 1 showed minimum inhibitory zone (10 \pm 0.03 mm) at 0.5 μ l concentration and maximum inhibitory zone (18 \pm 15 mm) at 2.0 μ l concentration. Similarly, the methanol extract of *P. amboinicus*– leaf on *S. pyogenes* SA 1 exhibited lower inhibitory activity (3 \pm 0.08mm) at 0.5 μ l concentration and higher inhibitory activity (10 \pm 0.01mm) at 2.0 μ l concentration. The distilled water extract of *P. amboinicus* on *S. pyogenes* SA 1

showed no inhibitory activity in distilled water at 0.5, 1.0 and 1.5µl concentration and exhibited inhibitory activity (2 ± 0.004 mm) at 2.0 µl concentration. In this study, a significant maximum zone of inhibition on *S.pyogenes* SA 1 was observed in the acetone extract of *P.amboinicus* when compared to the methanol extract and very low level of inhibitory zone was observed at distilled water extract (Plate 1, Table 1). According to previous reports, the herbal extracts with various polar solvents exhibited antimicrobial activity against various bacterial pathogens. The herbal plant *P.amboinicus* used as a therapeutically active agent for the treatment of various disease causing bacterial strains such as *S. aureus* (Hasani *et al.*, 2003), *S.epidermidis*, Enterococcus faecalis (Bugayong *et al.*, 2019), Aeromonascaviae (da Costa *et al.*, 2010), *E. coli* (Bugayong *et al.*, 2019), Pseudomonas aeruginosa (Manjamalai *et al.*, 2012), *Salmonella* sp. (Dao *et al.*, 2019), *Aspergillus niger* (Hasani *et al.*, 2003) and *C. albicans* (Manjamalai and Tom 2012) which were showed the inhibitory zone ranged from 17 to 42 mm with different concentration of extract was partially resembled with our present study. The main compounds of *P.amboinicus* such as phenol and flavonoids may be inhibited the bacterial growth of all the 35 strains of *Streptococcus* sp. (Swamy *et al.*, 2017; Silva *et al.*, 2020). Earlier studies on the methanol extract of marigold petal showed maximum inhibitory zone (18mm) on *S. aureus* was contrast to the present study (Efstratiou *et al.*, 2012).

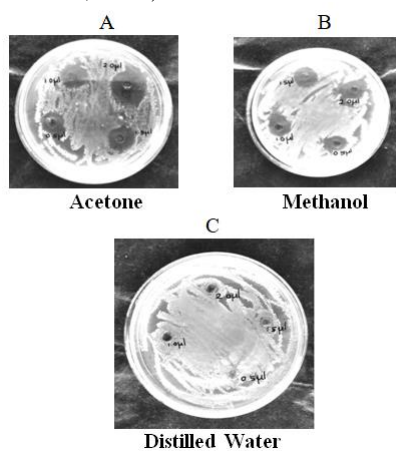


Plate 1. Zone of inhibition of the acetone, methanol and distilled water extracts of *P. amboinicus* – leaf against the silkworm pathogen *S. pyogenes* SA 1.

Table 1: Effect of *P. amboinicus*– leaf extract on *S. pyogenes* SA 1.

Sr. No	Concentration of extract (µl)	Zone of inhibition (mm)		
		Acetone extract	Methanol extract	Distilled water
1.	0.5	10 ± 0.03	3 ± 0.08	-
2.	1.0	12 ± 0.14	5 ± 0.11	-
3.	1.5	15 ± 0.09	9 ± 0.05	-
4.	2.0	18 ± 0.15	10 ± 0.01	2 ± 0.004

Phytochemical screening. The phytochemical screening of the crude extract of *P. amboinicus* – leaf with different solvents such as acetone, methanol and

distilled water indicated the presence of various Phytoconstituents. Both the acetone and methanol extract of *P.amboinicus* – leaf indicated the presence of alkaloids, flavonoids, coumarins, steroids, tannins, and carbohydrates, except glycosides and saponins. The distilled water extract of *P. amboinicus* - leaf also indicated the presence of alkaloids, flavonoids, glycosides, tannins and carbohydrates except coumarins, steroids and saponins (Table 2). The herbal plant *P. amboinicus* was a succulent perennial herb with aromatic pubescence and inherent healing power due to the presence of various phytochemical compounds (Arumugam *et al.*, 2016). These herbal plants synthesize secondary metabolites such as alkaloids, flavonoids, tannins, coumarins and glycosides with different molecular structures and few of them possess antimicrobial properties was showed resemblance with the present study (Lanciotti *et al.*, 2004).

Table 2: Phytochemical screening of crude extract of *P. amboinicus* - leaf in different solvents.

Sr. No.	Constituents	Acetone	Methanol	Distilled water
1.	Alkaloids	+	+	+
2.	Flavonoids	+	+	+
3.	Coumarins	+	+	-
4.	Steroids	+	+	-
5.	Glycosides	-	-	+
6.	Tannins	+	+	+
7.	Carbohydrate test	+	+	+
8.	Saponins	-	-	-

(+) present, (-) absent

GCMS analysis. The GCMS analysis of the acetone extract of *P. amboinicus* – leaf revealed the presence of 18 compounds (Table 3). The major compounds were detected in the acetone extract of *P.amboinicus* – leaf was 1,8- Nonadiyne (16.442%), Thymol (16.168%) 1, 3, 7 - Octatriene, 3, 7 - Dimethyl (12.020%), Phenol, 2 methyl - 5 - (1 - Methyl ethyl) – (10.969%), E - 2- Octadecadien - 1 - OL (8.402%), 1,2 - Benzene dicarboxylic acid, BIS (2- Methyl propyl) ester (4.759%), URS -12- ENE (4.743%), 1-Oxaspiro (2.5) OCT – 5-ENE, 8, 8 - dimethyl - 4 - methylene - (3.984%), 1- Hexacosanol (3.898%), 9, 19 - Cyclogest - 24 (28) - EN, 3 - OL, 4, 14 - Dimethyl -, Acetate, (3. Beta; 4. Alpha, 5. Alpha) - (3.318%), (1S, 4R) - P - Mentha - 2, 8 - Diene, 1 - Hydroperoxide (3.242%), Geranylgeraniol (2.673%) Hexadecane, 1- Chloro – (1.262%), Octadecanoic acid (2.020%), N-Hexdecanoic acid (1.872%), Stigmasterol (1.794%), Sigmastan - 6, 22 - Dien, 3, 5 - Dedihydro (1.298%), Hexatriacondane (1.134%) - (Table 3, Fig. 1). The GCMS analysis of the earlier studies revealed that the acetone extract of *P. amboinicus* possesses antimicrobial agents such as Phenol 1,2 - methyl -5-(1- methyl ethyl)-; thymol; (1S, 4R)- P -mentha - 2,8 -Diene, 1 - hydroperoxide; Octadecanoic acid; 1- Hexacosanol; and stigmasteran-6, 22 - dien, 3,5, - dedihydro- were showed resemblance with previous studies (Gurgel *et al.*, 2009).

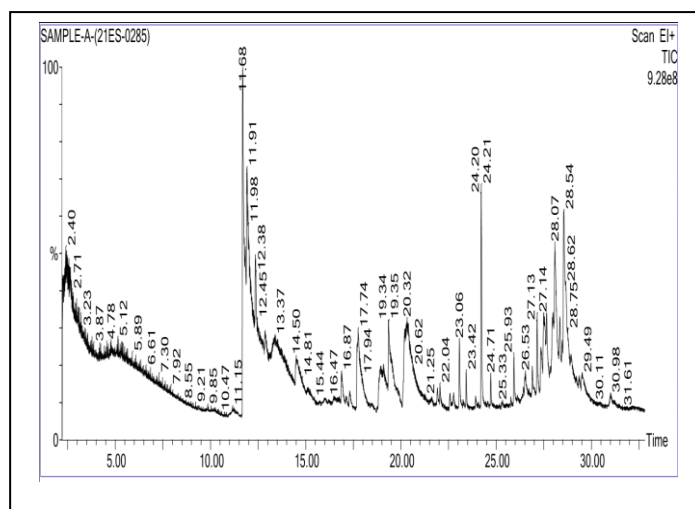


Fig. 1. Chromatogram of the acetone extract of *P. amboinicus* – leaf.

Table 3: GC-MS analysis of acetone extract of *P. amboinicus* – leaf.

Sr. No.	Name of the Compound	Retention time (min)	Area (%)	Molecular weight (g/mol)	Molecular formula	Properties
1.	Phenol 1,2 - Methyl - 5- (1- Methyl ethyl)-	11.682	10.969	150	C ₁₀ H ₁₄ O	Antimicrobial agent (Gurgel <i>et al.</i> , 2009)
2.	1, 8 – Nonadiyne	11.907	16.442	120	C ₉ H ₁₂	Antioxidant activity (Chun <i>et al.</i> , 2005)
3.	1, 3, 7 - Octatriene, 3, 7 – Dimethyl-	12.362	12.020	136	C ₁₀ H ₁₆	Antioxidant activity (Chun <i>et al.</i> , 2005)
4.	1-Oxaspiro (2.5) OCT - 5 ENE, 8, 8 - Dimethyl - 4- Methylene	12.873	3.984	150	C ₁₀ H ₁₄ O	Anticancer activity (Sathasivam and Elangovan 2011)
5.	Thymol	13.398	16.168	150	C ₁₀ H ₁₄ O	Antimicrobial activity (Gurgel <i>et al.</i> , 2009)
6.	(1S, 4R)- P - Mentha -2, 8 - Diene, 1- Hydroperoxide	14.498	3.242	168	C ₁₀ H ₁₆ O ₂	Antibacterial activity (Gurgel <i>et al.</i> , 2009)
7.	1, 2 - Benzene dicarboxylic Acid, BIS (2- Methyl Propyl) Ester	17.745	4.759	278	C ₁₆ H ₂₂ O ₄	Anticancer activity (Sathasivam and Elangovan 2011)
8.	N- Hexadecanoic acid	18.920	1.872	256	C ₁₆ H ₃₂ O ₂	Anti-inflammatory activity (Gurgel <i>et al.</i> , 2009)
9.	Octadecanoic acid	19.080	2.020	284	C ₁₈ H ₃₆ O ₂	Antimicrobial activity (Gurgel <i>et al.</i> , 2009)
10.	1- Hexacosanol	19.350	3.898	382	C ₂₆ H ₅₄ O	Antimicrobial activity (Gurgel <i>et al.</i> , 2009)
11.	E-2- Octadecadien - 1 – OL	20.316	8.402	268	C ₁₈ H ₃₆ O	Antioxidant activity (Chun <i>et al.</i> , 2005)
12.	Geranylgeraniol	24.212	2.673	290	C ₂₀ H ₃₄ O	Anti-inflammatory activity (Gurgel <i>et al.</i> , 2009)
13.	Hexatriacontane	27.143	1.134	506	C ₃₆ H ₇₄	Antioxidant activity (Chun <i>et al.</i> , 2005)
14.	Stigmasterol	27.489	1.794	412	C ₂₉ H ₄₈ O	Anticancer activity (Sathasivam and Elangovan 2011)
15.	Hexdecane, 1-Chloro-	27.649	1.262	260	C ₁₆ H ₃₃ Cl	Anticancer activity (Sathasivam and Elangovan 2011)
16.	Stigmastan - 6, 22 - Dien, 3, 5 - Dedihydro-	27.984	1.298	394	C ₂₉ H ₄₆	Antibacterial activity (Gurgel <i>et al.</i> , 2009)
17.	9, 19 - Cycloergost - 24(28) - EN-3-OL, 4-14- Dimethyl -, Acetate, (3. Beta., 4. Alpha., 5. Alpha)-	28.074	3.318	468	C ₃₂ H ₅₂ O ₂	-
18.	URS - 12- ENE	28.539	4.743	410	C ₃₀ H ₅₀	Antioxidant activity (Chun <i>et al.</i> , 2005)

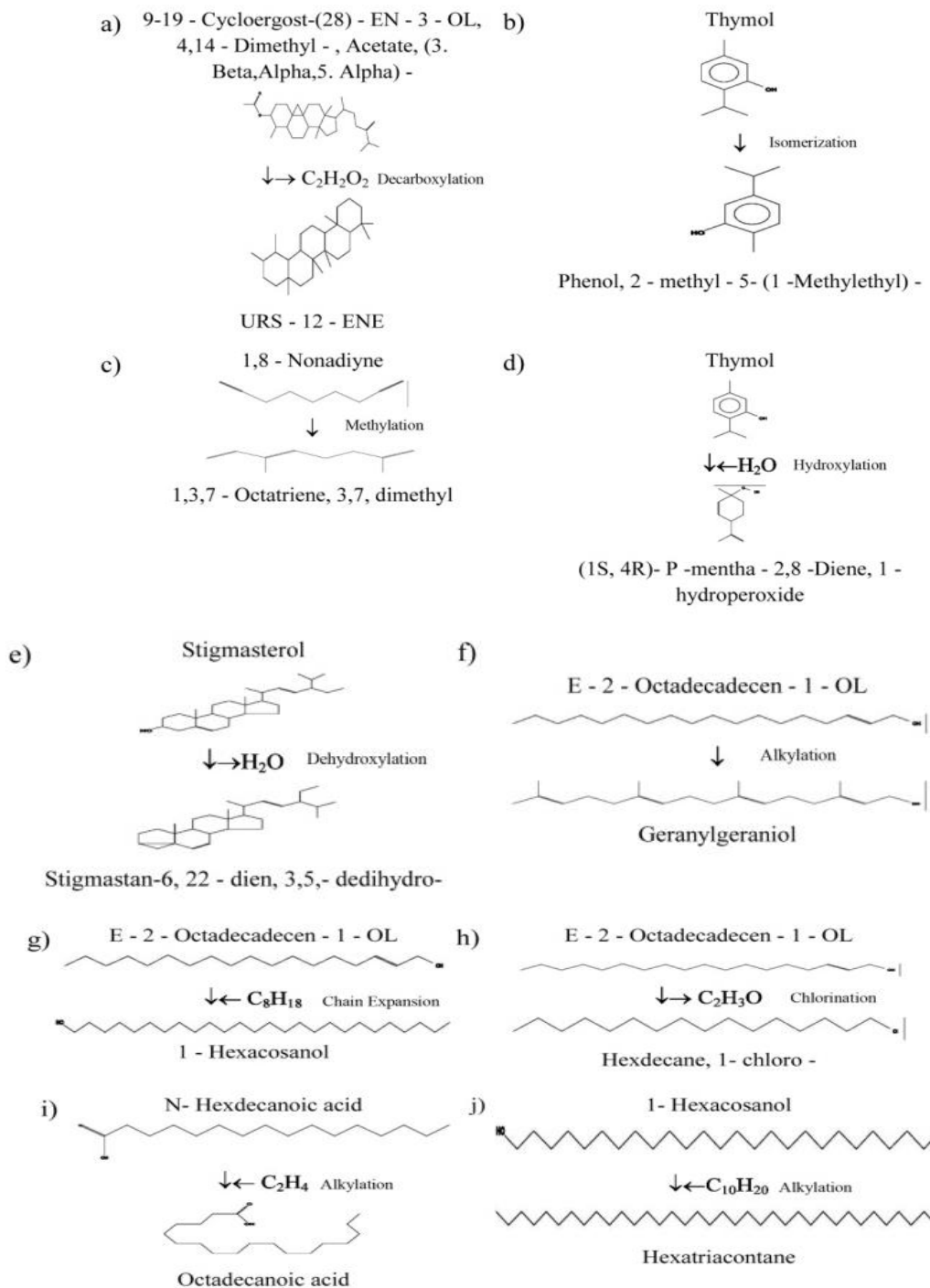


Fig. 2. Probable pathways for the internal conversion of major compounds in the acetone extract of *P.amboinicus* – leaf.

As per the previous GCMS report of (Chun *et al.*, 2005) the acetone extract *P. amboinicus* possesses an antioxidant active compound such as 1,8 - Nonadiyne; 1,3,7 - Octatriene, 3,7, dimethyl; E - 2 - Octadecadien - 1 - OL; Hexatriacontane; and URS 12- ENE; were in agreement with the sample analyzed in the present study.

These anti-microbial compounds could be playing a vital role in degradation of bacterial pathogen *S. pyogenes* SA 1. As per results obtained through GCMS analysis of the acetone extract of *P. amboinicus*, a probable internal conversion of major compounds to

minor compounds may be occurred which were illustrated as pathways.

The compound, 9-19 - Cycloergost-(28) - EN - 3 - OL, 4,14 - Dimethyl -, Acetate, (3. Beta, 4. Alpha, 5. Alpha) - may be undergo removal of $C_2H_2O_2$ and decarboxylation and ring opening reaction into URS - 12 - ENE. (Fig. 2a). The compound, Thymol may be internally converted into Phenol, 2 - methyl - 5- (1 - methyl ethyl) - due to the process called stereo isomerization (Fig., 2b). The compound 1,8 - Nonadiyne may be under go methylation and transform into 1,3,7 - Octatriene, 3,7, dimethyl (Figure, 2c). The compound Thymol may be undergo addition of H_2O

into (1S, 4R)- P -mentha - 2,8 -Diene, 1 - hydroperoxide due to the process is called hydroxylation and resonance breakdown (Fig. 2d). The compound Stigmasterol may be undergo the removal of H₂O and dihydroxylation followed by formation new bicyclic ring and internally converted into Stigmastan-6, 22 - dien, 3,5,- dedihydro- (Fig. 2e). The compound E - 2 - Octadecadien - 1 - OL may be undergo two carbon alkylation and converted into Geranylgeraniol (Fig. 2f). The compound E - 2 - Octadecadien - 1 - OL may be under go addition of C₈H₁₈ and chain expansion and transform into 1 -Hexacosanol (Fig. 2g). The compound E - 2 - Octadecadien - 1 - OL may be internally change into Hexdecane, 1- chloro -due to the process called chlorination with removal of C₂H₃O (Fig., 2h). The compound N- Hexdecanoic acid may be internally converted into Octadecanoic acid due to the process called alkylation chain extension with crown model and addition of C₂H₄ (Fig. 2i). The compound 1-Hexacosanol may be internally transform into Hexatriacontane and this process are alkylation and addition of C₁₀H₂₀ with removal of oxygen (Fig. 2j).

The results of the present study also revealed that the acetone extract of *P. amboinicus* possess an anti-cancer active compound such as 1 - Oxaspiro (2.5) OCT - 5ENE, 8, 8 -dimethyl - 4 -methylene; 1,2 Benzene dicarboxylic acid, BIS (2 -Methyl propyl) ester; stigmasterol; and Hexdecane, 1- chloro which were in accordance with the sample analyzed in the previous studies (Sathasivam and Elangovan 2011).

The results of the GCMS analysis on the acetone extract of *P. amboinicus* showed anticancer and anti-inflammatory effect due to the presence of Hexadacane, Stigmasterol and Geranylgeraniol compounds. The previous studies on the crude hydroalcoholic extract of *P. amboinicus* leaves also found to possess an anti-inflammatory activity (Hamalainen, 2007; Gurgel *et al.*, 2009). A significant anticancer effect was observed in the tumor cells due to the presence of compounds in the hydroalcoholic extract of *P. amboinicus* leaves (Akagi *et al.*, 1995; Yang *et al.*, 2000).

CONCLUSIONS

P. amboinicus is an important aromatic medicinal herb packed with many bioactive constituents and nutrients, which are important for maintaining good health. The biological properties are attributed to the occurrence of a wide range of bioactive compounds in *P. amboinicus*-leaf extracts which possess promising anti-microbial effects. The GC-MS studies of the acetonic extract of *P. amboinicus* – leaf was showed various compounds. Further study is required to find out the accurate compound responsible for the plants medicinal value. The major components of nonadiyne are used in biological activities.

FUTURE SCOPE

P. amboinicus is an important medicinal plant with antimicrobial, anti-inflammatory effect. It is effective against various bacteria. This plant possesses several unknown bioactive properties that provide opportunities for additional research.

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Conflict of Interest. None.

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