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Essential oil of *Aganosma dichotoma*: Extraction, Phytochemical and antimicrobial analysis.

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Abstract **- To ascertain the essential oil Phytochemical and to test for the antibacterial properties of aqueous and ethanolic crude extract Aganosma dichotoma flower. The extraction and Phytochemical screening of essential oils was done by using soxhlet extraction and hydro-distillation using the Clevenger apparatus, and analysis performed by gas chromatography equipped with a flame ionization detector (GC-FID). Phytochemical characterization of essential oils showing positive results for tannins, glycosides, terpenes, nitrogenous compounds, Alkaloids, non protein amino acids, cyanogenic compounds, flavonoids. The presence of phytochemical compounds in the essential oils shows a significant effect on pathogenic bacterial strains and they also showed significant antioxidant potential. Antibacterial activity and the minimum inhibition rate of essential oils from the flowers of Aganosma dichotoma were determined using the agar gel disc diffusion method against the pathogenic bacteria strain *Escherichia coli.***

Key words **- Essential oils, Aganosma dichotoma, soxhlet extraction, antibacterial analysis and Phytochemical screening.**

1. INTRODUCTION

Herbs are widely distributed and used as traditional medicines [1]. The curative properties are well documented. From different studies, the evidences are found that some antibacterial agent and they even act against some antibiotic resistant strains. *Aganosma dichotoma* (Apocyanaceae) commonly known as Malati, a big, woody climber which is a jasmine like flowers, native to Indian subcontinent, China and Southeast Asia. The Apocyanaceae family is one of the 10 largest family having medicinal plants. It have 392 genera and 5140 species all over the world. These are found in various tropical and subtropical regions [2]. In India we can found 30 genera and 60 species throughout Bihar, Uttar Pradesh, Madhya Pradesh, Mizoram, Odisha, West Bengal, Andhra Pradesh, Kerala. Over the last few years the demand of medicinal herbs are highly increased due to vast chemical diversity for which the quality, safety and efficiency of herbs needed to be ensured [3]. By standardizing the herbs, we can maintain the quality of the material which can be used for treatment of various disease and disorder.

Essential oils are liquid aromatic compounds and volatile in nature. These are form in the chloroplast of the leaf or vesinogenous layer of cell wall [4]. They are found in different parts of plant such as leaves, seeds, flower, berries, rhizomes, root, bark, petals, resins etc. Essential oil extracted from different parts of the plant may have different scents and properties. The quantity of extracted essential oil is determined by some factors such as climatic geographical and seasonal conditions. The essential oil are also known as chemical weapon because it protects the plants from various insects fungal or bacterial attacks. They are also act as plant pheromones [5]. These are also used in various therapeutic properties. These properties can be verified after the extraction of oil from plant materials.

These are biological active, naturally occurring chemical compounds which provide medicinal benefits for humans. They protect plants from damage and various diseases also contribute to the plant color, flavor and aroma. The plant chemicals that protect plants from environmental hazards as pollution, drought, stress, pathogenic attack, UV exposure are called as phytochemicals. These are classified as primary or

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secondary metabolites depending upon their role in plant metabolism. Primary metabolites are the sugars, amino acids, proteins, purine and pyramidine of nucleic acid, chlorophyll etc. Secondary metabolites are the plant chemicals such as alkaloids, terpenes, flavonoids, lignin's, plant steroids, saponins, phenolics and glucosides.

Nature has been a source of medicinal agents for 1,000 of years and impressive no of modern drugs has been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine [6].

Many works have been done which aim at knowing the different antimicrobial and photochemical constituents of medicinal plants used them for treatment of microbial infection (both tropical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious micro organisms have become resistance. During the last 10 years, the pace of development of new antimicrobial drug has slowed down while the prevalence of resistance (especially multiple) has increased astronomically [7].

In this study we aim to extract the essential oils present in the flowers of Aganosma dichotoma, and their Phytochemical screening and antimicrobial analysis.

2. MATERIALS & METHODS

Collection of plant materials: The fresh plant parts of *Aganosoma dichotoma* such as flowers were collected from a plant growing at the Medicinal garden of Biotechnology department, GIET University, Gunupur, Odisha. The plant was authenticated again at the Dept. Life science, GIET University, Odisha.

Figure 1: *Aganosma dichotoma* flower plant

Extraction of Plant materials: Materials which are required for extraction are Plant flowers, Scissor, Mechanical grinder, Whatman filter paper no 1, Soxhlet extractor, distilled water, Ethanol, Dimethyl sulfoxide (DMSO)

Healthy flowers were collected and washed with tap water followed by distilled water to remove the soil & dirt particles . The flowers were cut into small pieces & then dried at shade. Utmost care had been taken to avoid growth of any fungal particles on flowers. The dried flowers were powdered separately by a mechanical grinder. The flower powder (5 grams) was packed in filter paper carefully. The extraction was made in 50 ml of Distilled water at 100^0C for 6 hours by using Soxhlet extractor. Similarly ethanol extraction was made at 60^0 C for 6 hours by using Soxhlet extractor. The extracts were then filtered through a Whatman no 1 filter paper and then dried at 37^0 C. The alkaloids were stored at 4^0 C for future use. The stock solution is prepared by dissolving 1 gram of each extract in 5 ml of Dimethyl sulfoxide (DMSO).

Figure 2: Soxhlet extraction of essential oil

ANALYSIS OF PHYTOCHEMICALS

Standard preliminary Phytochemical analysis methods were followed for testing the presence of Carbohydrates, phytosterols, proteins, saponins, Phenolics compounds and Tannins

Determination of Antimicrobial Activity and MIC

2.4.1 Collection of Bacterial strain: Bacterial strain, *E.coli* MTCC 78 is obtained from Institute of microbial Technology (IMT), Chandigarh, India. Then the bacterial strain was subjected to broth culture at the laboratory. The media used for broth culture as per the recommended by IMT.

*Preparation of Nutrient agar media***:** Beef extract- 1 g, Yeast extract-2 g, Peptone-5 g, NaCl-5 g, Distilled water -1lit

2.4.2. *Antimicrobial activity assay:* Sterilized nutrient agar plates are prepared by pour plate method. To the nutrient agar medium 0.1 ml bacterial culture was transferred and spread with a sterile bent glass rod. The gel was puncture with the help of gel puncher (5mm diameter). Then the wells were added within different concentrations of plant extracts. Then the petridish were incubated at 37° C in normal position for 48 hours. The zones of inhibition were determined using scale & results are expressed in mm.

Figure 3: *Escherichia coli* MTCC78 bacterial strain.

2.4.3 Minimum inhibitory concentration (MIC): flower extracts (1 gm of each) was dissolved in 8 ml of sterile Muller Hilton Broth which yield an initial concentration of 125 mg/lit. Subsequently, by following two fold serial dilutions the concentrations such as 125, 62.5, 31.25, 15.65, 7.83, 3.91, 1.95, 1.00, 0.50, 0.25, 0.13 mg/lit were prepared. One ml of standardized inoculums of test organism was introduced in to the extract –nutrient broth mixture and then incubated at 37° C for 24 hour. The lowest concentration of the extract that inhibited the test organisms was recorded as minimum inhibitory concentration (MIC).

3. RESULT AND DISCUSSION

3.1. *Essential Oil:* The yield of essential oil from the flower of *Aganosma dichotoma* was $0.8 \pm 0.13\%$ based on the fresh weight of the sample. The phytochemical profile of the essential oil components from the flowers of Aganosma dichotoma as shown in the Table 1 is composed of fourteen compounds with three unidentified; ethyl valerate, decane, ethyl lactate, (*Z*) 4-decenal, hexyl hexanoate, (−)-*γ*-elemene, geranyl butyrate, humulene oxide, 4-propyl-guaiacol, uncineol, eicosane, tetradecanol, and acetovanillone. The main constituents in the *Aganosma dichotoma* essential oil were uncineol 30.9%, eicosane 27.4%, eicosane 21.6%, and 4-propyl-guaiacol 14.05%.

Table 1: Chemical composition identified in the essential oils of flowers of Aganosma dichotoma

S. No	Chemical composition of *Aganosma dichotoma*	Percentage concentration
1	Ethyl valerate	--
2	Decane	--
3	Ethyl lactate	--
4	(Z)-4-decenal	0.05
5	Hexyl hexanoate	1.19
6	c-elemene	0.16
7	Geranyl butyrate	0.5
8	Humulene oxide	2.7
9	4-Propyl-guaiacol	14.05
10	Uncineol	30.9
11	Eicosane	27.4
12	Tetradecanol	4.7
13	Acetovanillone	0.9

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Phytochemical analysis: Phytochemical screening of the essential oil from the flowers of *Aganosma dichotoma* showing the presence of some of the secondary metabolites as shown in the Table 2.

Table 2: Phytochemical constituents of aqueous and ethanolic flower extracts of *Aganosma dichotoma*.

Phytochemical constituents	Aqueous extract	Ethanolic extract
Reducing Sugar	-	++
Terpenoids	-	-
Phenolics	+	+
Anthraquinone	-	-
Steroids	-	-
Flavonoids	-	+
Saponins	+	+
Glycosides	+	+
Tannins	+	+
Alkaloids	+	+
Nitrogenous compounds	-	+

The Phytochemical analysis results showed that the presence of Phenolics, Saponins, Glycosides and Tannins only showing positive result with aqueous extract. Whereas the ethanolic flower extract of *A. dichotoma* showing reducing sugars, phenolics [8], flavonoids, saponins, glycosides, tannins [9,10], alkaloids [11] and nitrogenous compounds [12].

Antimicrobial analysis:

Minimum Inhibitory Concentration (MIC): *E. coli* were cultured in a medium and both the extract of flower was applied to find the efficacy of antimicrobial activity. However both the aqueous and ethanol extract showing antimicrobial property. More over the ethanol extract is more effective then aqueous. The zone of inhabitation of ethanol extract was found 1.5cm and aqueous was 1.0cm respectively in *E .coli* as shown in Table 3 and Figure 4.

Table 3: Zone of inhibition of essential oil of aqueous and ethanolic extracts of Aganosma dichotoma against *E.coli* bacteria

Test Organism	Flower Extract			
	Control		Test	
	Aqueous	Ethanol	Aqueous	Ethanol
E .coli	**0**	**0**	**1.0**	**1.5**

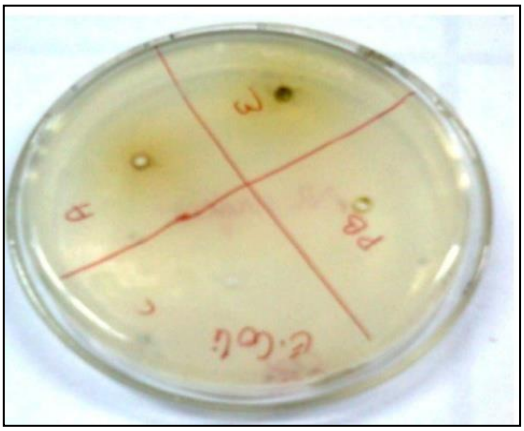

Figure 4: Zone of inhibition of essential oil of aqueous and ethanolic extracts of Aganosma dichotoma against *E.coli* bacteria

The Minimum Inhibitory Concentration (MIC) of both the extracts were analyzed against E .coli. It was observed that the Ethanol extract has shown MIC at 10mg/ml, and aqueous extract has shown MIC at 20mg/ml respectively as shown in table 4.

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Table 4: Minimum Inhibitory Concentration of aqueous and ethanolic extracts of Aganosma dichotoma

Test Organism	Flower Extract Concentration					
		5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
E .coli	Ethanol	+ve	-ve	-ve	-ve	-ve
	Aqueous	+ve	+ve	+ve	-ve	-ve

+ ve = Growth - ve = No Growth

CONCLUSION

As per the results its concluded that the ethanolic solvent extract showing significant Phytochemical constituents when compared with aqueous flower extract in case of Aganosma dichotoma. The essential oils of this flower also showing presence of Phytochemical constituents like tannins, glycosides, terpenes, nitrogenous compounds, Alkaloids, non protein amino acids, cyanogenic compounds, flavoinds. The essential oils also showing significant antimicrobial Property against *E .coli* and ethanol extract was found more effective than aqueous extract.

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