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Studies on antioxidant and antimicrobial activity of *Acorus calamus* and *Myristica fragrans*

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Abstract
Acorus calamus & *Myristica fragrans* extracts are both used in this study. These have medicinal uses and commonly used as Antidepressant. The Phytochemical screening and antimicrobial assay of both the plants indicated positive application these plant extracts for medicinal use wherein their antioxidant property is an added advantage. The presence of tannins aid in precipitation of cells especially microbes and can be used as antimicrobial agents.

Keywords: Antioxidant, Antimicrobial

1. Introduction
Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Over one and a half million practitioners of the Indian system of medicine use medicinal plants in preventive and curative applications. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as source of medicinal agents [1]. The World Health Organization (WHO) has given guidelines to the member states to ensure about genuine use of plants and their parts before their use for human health [2].

Acorus calamus & *Myristica fragrans* extracts are both used in this study. These have medicinal uses and commonly used as Antidepressant. The many number of medicinal plants are used in the treatment of cellular and metabolic diseases such as diabetes, obesity and cancer etc. There are some speculations that the generation of free radicals inside the body in some physiological condition is resulted in cellular changes and development of cancer etc and this could be neutralized by the antioxidants from different medicinal plants. Several studies have shown that plant derived antioxidant nutraceuticals scavenge free radicals and modulate oxidative stress related degenerative effects. Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging. The recent advances in research suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular disease and cancer. The free radical neutralizing property of several plants was reported by previous studies. The extracts from number of medicinal plants which are known o have some biologically active principles are used in ayurvedic preparations and these extracts are prepared in bulk for commercial purpose.

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals. Oxygen hydroxyl radicals (OH) and non-free radical species such as water and singlet oxygen re various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process. In foods, ROS can cause lipid peroxidation, which leads to the deterioration of the food. The addition of antioxidant is a method for increasing shelf life foods. Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity [3].

The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. In addition, they have a m*etal* chelation potential [4]. The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and there by improve the quality and nutritional value is also raising interest among scientist, food manufactures and consumers due to functional food with specific health effects [4]. Most of plants are resistant to microbes and it is generally only specially is the first organisms that have evolved the capacity of defenses, so that many pathogens have a narrow host ranges. The first passive barrier that defends plants against microbes is the wax layer present on the cuticle of leaves and fruits, phytoanticipins, phytoalexins like tannins suberins and ligins. This defense mechanism against pathogen is called antimicrobial activity [5].

Plants do not have immune system directly comparable with that of animal's [6].Thus to protect themselves from infection by a variety of pathogens plants have evolved a host of defense mechanism includes the host of defense mechanism [7]. Class4 includes acidic proteins with molecular masses of 13-14Kda that have Sequences similarity with the sweet protein thaumatin from *Thaumatococcus daniellii*. In addition these PR proteins induced by pathogen attack or stress, other families of plants antifungal proteins, lipid –transfer proteins and chitin binding proteins [8]. Simple phenols and phenolic acids: some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic propane derived compounds which are in highest oxidation state. The destruction or modification of tannin with time plays an important role in the ripening of fruit and ageing of wine [9].

Acorus calamus, a "sweet flag" and "calamus" other common names include beewort, bitter pepper root, calamus root, flag root, gladdon, myrtle flag, myrtle grass, myrtle root, myrtle sedge, pine root, rat root, sea sedge, sweet cane, sweet cinnamon, sweet grass, sweet myrtle, sweet root, sweet rush, and sweet sedge. Common names in Asia include: "shoubu " (Japanese); "vacha"; "changpo " (Korean); "bacch" (Unani); "bajai", "gora-bach", "vasa bach" (Hindi); "vekhand" (Marathi); "vasambu" (Tamil); "vadaja", "vasa" (Telugu); "baje" (Kannada); "vayambu" (Malayalam); Haimavati, "bhutanashini", "jatila" (Sanskrit) and "bojho" (Nepali) [10-11].

The name *sweet flag* refers to its sweet scent and its similarity to *Iris* species, which are commonly known as flags in English since the late fourteenth century. There are three cytotypic forms distinguished by chromosome number: a diploid form (2n=24), an infertile triploid form (2n=36), and a tetraploid form (see below). The triploid form is the most common and is thought to have arisen relatively recently in the Himalayan region through hybridization of the diploid with the tetraploid Probably indigenous to most of Asia, the triploid form *Acorus calamus* var. *calamus* (also known as var. *vulgaris* or var. *verus*) has now been introduced across Europe, Australia, New Guinea, South Africa, Reunion and North America The tetraploid form *Acorus calamus* var. *angustatus* is native throughout Asia, from India to Japan and the Philippines and from Indonesia to Siberia. The diploid form *Acorus americanus* or *Acorus calamus* var. *americanus* is found in northern subarctic North America and scattered disjunction areas throughout the Mississippi Valley, and furthermore diploids are also found in Mongolia, central Siberia (Buryatia), Gilgit–Baltistan in Pakistan (claimed by India) and northern Himachal Pradesh in India. It is extinct in some parts of the United States and Canada. It may not have been native to some of these areas. Pre-Columbian populations are thought to have dispersed it across parts of the United States [12]. According to Heng Li, Guanghua Zhu and Josef Bogner in the Flora of China there is clear overlap in these characteristics and the different cytotypes are impossible to distinguish morphologically [13].

Triploid plants are infertile and show an abortive ovary with a shriveled appearance. This form will never form fruit (let alone seeds) and can only spread asexually. A further hexaploid form exists in central and northwestern Yunnan and Kashmir. This form has not been given taxonomic status. At least 3 different karyotypes have been classified as hexaploid; 2n=66in Yunnan and 2n=54 and 2n=72 in Kashmir Diploid plants in North America apparently produce no or only trace amounts of b-asarone. According to one study, triploids produce a small amount, constituting around 0.3% of the rhizome in crude content, whereas tetraploids may be found in at least two chemotypes, one with 2.0%, and one with 4.0 to 8.0% [14-17].

A. calamus has been an item of trade in many cultures for thousands of years. It has been used medicinally for a wide variety of ailments, and its aroma makes calamus essential oil valued in the perfume industry. The essence from the rhizome is used as a flavor for pipe tobacco. It is also used in bitters In Lithuania *Ajeras* (Sweet flag) is added to home baked black bread [14]. The Potawatomi people powdered the dried root and placed this up the nose to cure catarrh [16]. *Myristica fragrans* was given a binomial name by the Dutch botanist Maartyn Houttuyn in 1774. It had earlier been described by Georg Eberhard Rumphius, among others the specific epithet *fragrans* means "fragrant" [18]. *Myristica fragrans* are used for diarrhea, nausea, stomach spams and pain and intestinal gas. They are also used for treating cancer, kidney disease and insomnia. It is also used as fragrance in soaps and cosmetics.

The objectives of the study is for Proximate analysis' of crude extracts of *Acorus calamus* and *Myristica fragrans,* Analysis of tannin from extract of *Acorus calamus* followed by evaluation the antimicrobial activity of extracts of *Acorus calamus* and *Myristica fragrans.* And to screen the extracts of *Acorus calamus* and *Myristica fragrans* for their antioxidant property.

2. Material and methods

2.1 Preparation of extract

Powdered form of *Acorus calamus* & *Myristica fragrans* each of 4gm were taken and incubated in 40ml of Chloroform and kept overnight. Then, it is filtered and supernatant is collected and kept for evaporation and dissolved in same solvent. The filtrate is again taken and incubated in 40mL of Di ethyl ether and the procedure is repeated. Similarly the filtrate is again taken and incubated in acetone and methanol and all the extract was subjected to Phytochemical analysis.

2.2 Antimicrobial activity

Antimicrobial activity was determined by disc fusion method. Nutrient broth was prepared and sterilized at 121degree Celsius and 15lbs pressure and different cultures (*Escherichia coli, Streptococcus, staphylococcus aureus, pseudomonas aeruginosa, Bacillus subtilis, Fluorescent)* were inoculated under sterilized condition and incubated at 37 degree Celsius for 16 hours. This culture was further used.

2.3 Antioxidant activity

2.3.1 Di-Phenyl 2-Picryl Hydrazine (DPPH) Radical Scavenging Assay:

DPPH radical scavenging activity was performed by the Schimida *et al*, and wang *et al* method [19]. The reaction mixture the plants extracts, ascorbic acid, methanol and DPPH. The reaction mixture without DPPH was considered as blank. Then incubated at 37 degrees Celsius for 30 minutes and O.D was measured at 517nm.

The radical scavenging activity was measured as the decrease in the absorbance of DPPH and calculated using the following equation:

$$\text{Scavenging effect \%} = \frac{\text{Absorbance of sample}}{\text{1- Absorbance of control}} * 100$$

2.3.2 Analysis of tannin by thin layer chromatography

During phytochemical screening tannin was only present in crude extract of *Acorus calamus* and acetone extract of *Acorus calamus* [21].

An aliquot of extract is dissolved in 1 ml of appropriate solvent system

Chloroform: Methanol: Acetic acid (18:1:1)..Rf value was calculated.

2.3.3 Determination of total tannins present in acetone extract of *Acorus calamus*

Weigh 10mg of Gallic acid. Then dissolved in distilled water and paid back the volume up to 10 ml to obtain a level of 1 mg /ml as stock and the stock solution pipetted their 0.2,0.4,0.6,0.8,1 mg was added to 0.2 ml of folin ciocalteu. (After that diluted with D.H20 1:1) homogeneously mixed for 10 sec and then allowed to stand for 5mins.Then paid back the volume to 5ml with D.H20.Allow to stand for 95 mins and turn into blue color and measured on colorimeter by 517nm. [23]. The results were calculated using this formula:

$$\text{Total tannin content} = \frac{GAE*V*D*10^6}{W} *100$$

(GAE- Gallic acid equivalent, V- volume of sample, D-dilution of factor, W- weight of sample)

2.3.4 Bate Smith, Hagerman and Butler method.

The method Bate smith is based on reaction of tannin with the protein

Hemolyzed blood and colorimeter determination of residual hemoglobin. This method requires freshly drawn blood the commercial preparation of hemoglobin is unsatisfactory. Freshly drawn blood and dilute 50 fold with Distilled water. To 1 ml of this blood add 1ml of extract containing 0.3-08g of tannic acid. The precipitates is centrifuged the color is measured at

578nm and compare it with control. The results are expressed as relative astringency that is the ratio of concentration tannic acid to that tannin producing the same extent of precipitates. The relation between absorbance of hemoglobin remaining in the wide range of tannin concentration and for several times [22].

3. Results

3.1 Phytochemical screening of *Acorus calamus* and *Myristica fragrans.*

Table 1: Phytochemical screening *of Myristica fragrans* in different solvents

Test	Chloroform	Di ethyl ether	Acetone	Methanol	Crude
Glycosides	-	-	-	-	-
Steroids	-	-	-	-	-
Tannins	-	-	-	-	-
Alkaloids	+	-	+	-	+
Flavonoids	-	-	-	-	-
Saponins	-	-	-	+	-
Quinones	-	-	-	-	-
Protein	-	-	-	-	-
Sugar	-	-	-	-	-

Table2: Phytochemical screening for *Acorus calamus* in different solvents

Test	Chloroform	Di ethyl ether	Acetone	Methanol	Crude
Glycosides	+	-	+	-	-
Steroids	-	-	-	-	-
Tannins	-	-	+	-	+
Alkaloids	-	-	+	+	-
Flavonoids	-	-	-	-	-
Saponins	-	+	-	-	-
Quinones	-	-	-	-	-
Protein	-	-	-	-	-
Sugar	-	-	-	+	-

3.2 Antimicrobial activity of *Acorus calamus* and *Myristica fragrans.*

The antimicrobial activity was done for all extracts of *Acorus calamus* and *Myristica fragrans*.

Table 3: Zone of Inhibition was observed in acetone extracts of *Acorus calamus* and *Myristica fragrans*.

Concentration (Acetone extract of *Myristica fragrans*) in µg	Zone of Inhibition					
	E. coil (cm)	*B.subtilis (cm)*	*Pseudomonas (cm)*	*Micrococcus (cm)*	*Proteus (cm)*	*S. Aureus (cm)*
100	1.4	0.6	0.2	0.2	0.4	0.6
200	0.4	0.4	1.0	1.0	1.0	1.4
300	1.8	0.2	0.6	1.8	0.2	0.2
400	0.2	0.2	1.6	0.8	1.6	0.8

Table 4: In acetone extract of *Acorus calamus* the antimicrobial activity was observed only in *E. coli* Strain

Concentration (Acetone extract of *Acorus calamus* in µg)	Zone of Inhibition *E. coli* (cm)
100	1.0
200	1.2
300	1.4
400	1.0

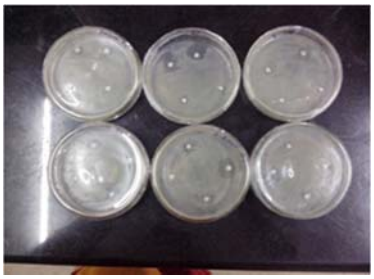


Fig 1

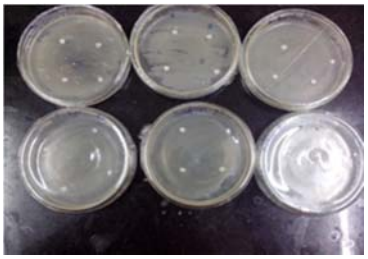


Fig 2

3.3 Antioxidant activity of *Acorus calamus* and *Myristica fragrans.*

The antioxidant activity of given sample was done by DPPH radical scavenging.

The antioxidant activity of *Acorus calamus* in different solvents was done by DPPH method.

The graphs are given below:

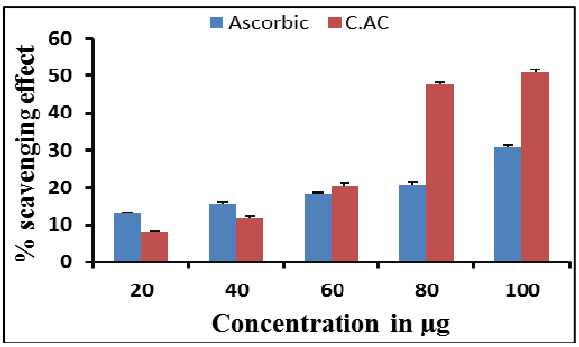


Fig 3: The antioxidant of chloroform extract.

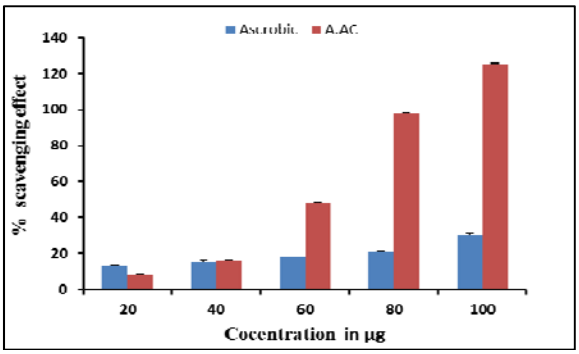


Fig 4: The antioxidant activity for Acetone extract

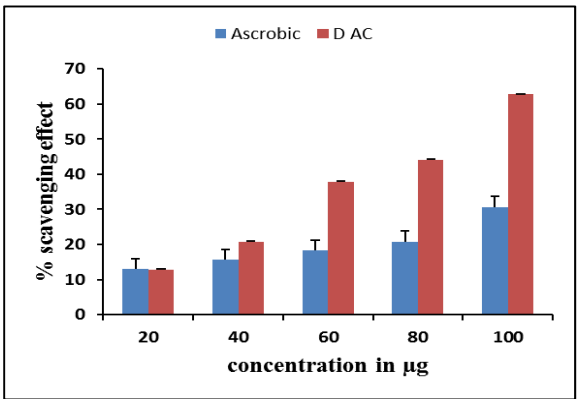


Fig 5: The antioxidant activity of Di ethyl ether extract.

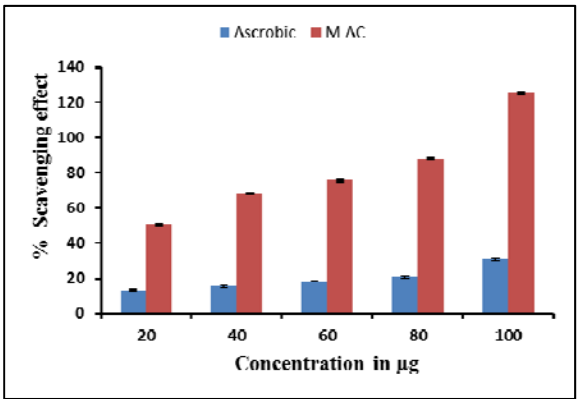


Fig 6: The antioxidant activity of Methanol extract.

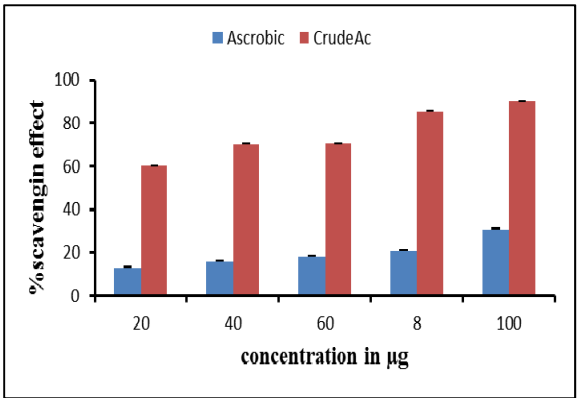


Fig 7: The antioxidant activity of Crude extract

The antioxidant activity of *Myristica fragrans* in different solvents was done by DPPH method.

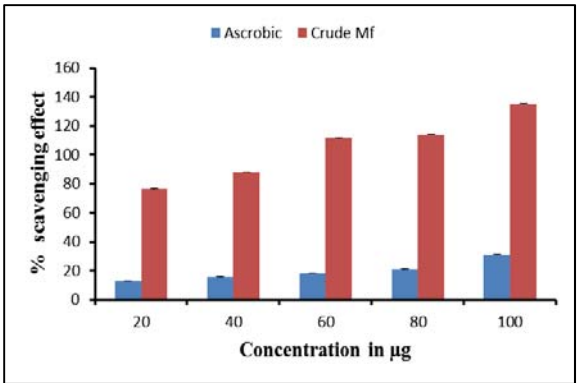


Fig 8: The antioxidant activity of Crude extract

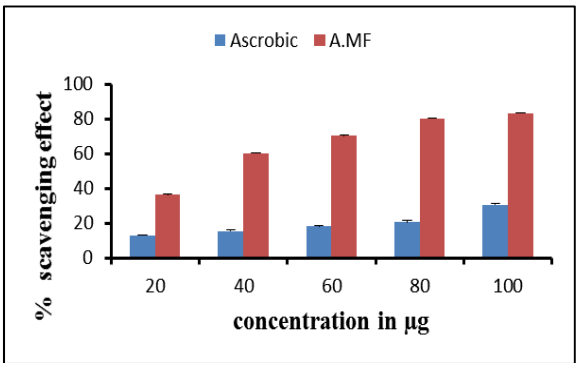


Fig 9: The antioxidant activity of Acetone extract

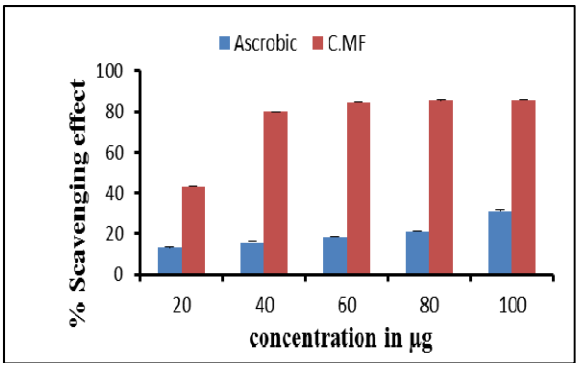


Fig 10: The antioxidant activity of Chloroform extract.

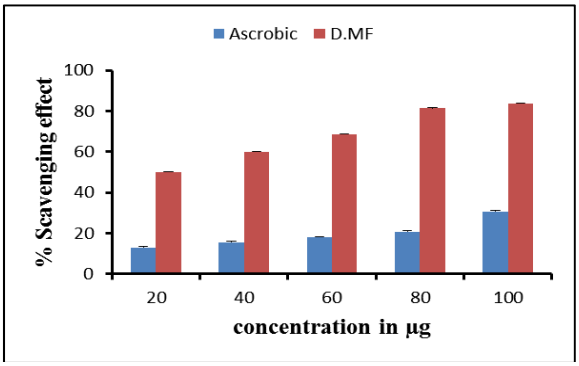


Fig 11: The antioxidant activity of Di ethyl ether extract.

3.4 Analysis of tannin by TLC.

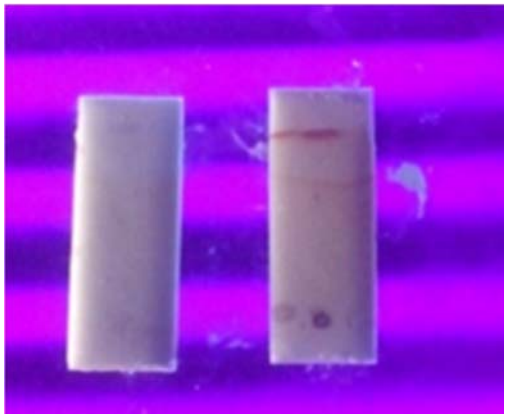


Fig 13: Tannin traces were found by doing TLC. It was observed in UV chamber.

3.5 Determination of total tannins present in plant extract

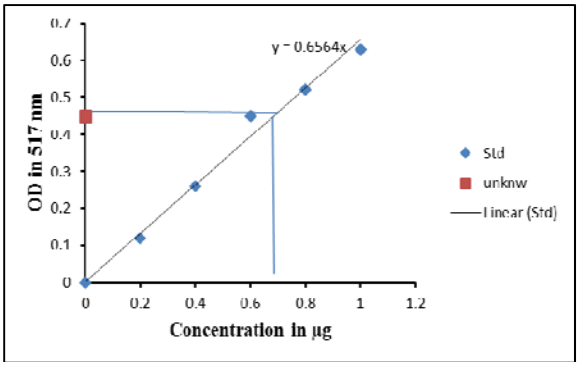
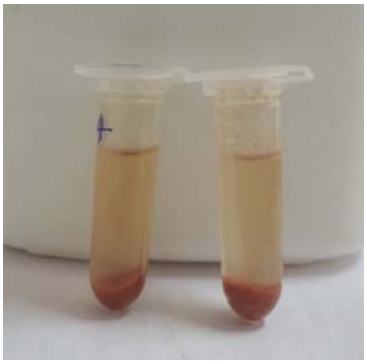


Fig 14: Total tannin present in given sample is $4.9*10^6$

Table 5: Bate smith, Hagerman and Butler.

	Blood + Sample	Blood
Blood with anticoagulant	1.033	1.680
Pure blood	0.974	2.148



Protein was precipated by this method. Tannin is more supernatant is clear and pellet is more. Here, blood with anticoagulant shows less tannin content. The blood without anticoagulant and sample content shows more tannin the supernatant is clear.

4. Discussion

Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of disease. Most of the plants have antioxidant property. DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents then losing color stoichiometrically with the number of electrons consumed which is measured spectrometrically at 517nm.Ascorbic acid is a potent free radical scavenger [23-26]. The chloroform extract of *Acorus calamus* showed more antioxidant property than any other solvent extract. AS showed in Fig 3.This shows that chloroform extract is potent DPPH free radical scavenger. According to the World Health Organization, medicinal plants would be the best source to a variety of drugs. About 80% of individuals from developed counties use natural medicine. Most plants are resistant to most microbes and it is generally only is first organisms that have evolved the capacity to overcome these defense, so that many pathogen have narrow host ranges. According to the World Health Organization, medicinal plants would be the best source to a variety of drugs. About 80% of individuals from developed counties use natural medicine.

Antimicrobial activity of *Acorus calamus and Myristica fragrans* of different solvents was studied. The Disc diffusion method was preferred to this study. Acetone extract of *Acorus calamus* and *Myristica fragrans* showed antimicrobial activity. The Table 3and 4 clearly shows the Zone of inhibition. While comparing with the other studies the acetone extract of the plant shows more activity than any other solvent and mainly show in *E.coli* strain. Many phenolics, such as alkaloids, tannin in particular have antioxidant capacities, because they have been found to posses' antioxidant and free radical scavenging effect. Preliminary phytochemical tests for *Acorus calamus* also showed the presence of tannin. Tannin was present in *Acorus calamus* and acetone extract of *Acorus calamus*. The presence of tannin was average, the experiments done showed presence of tannin. TLC was done for detection of tannin and spot was detected when observed under U.V spectrophotometer. It can be seen in Fig: 13. Determination of total tannin was done by using Gallic acid as standard. So that the total amount of tannin present in the acetone extract can be determined. In Fig: 14 the standard graph explain the total amount of tannin and it was calculated. The amount of tannin present was calculated. The phenolic compounds of plants have the ability to precipitate protein when treated with blood. Here we use Bate Smith, Hagerman and Butler method to precipitate protein and protein was precipitated as shown in Fig: 15.Tannin is important for leather industries hence finding different source for tannin could help this industries[9]. The present study was designed to obtain preliminary information on antimicrobial property and antioxidant property of *Acorus calamus* and *Myristica fragrans*. These plants have medicinal property, they are antidepressant and used for treatment of digestive disorders. It is also used for perfume industry to give fragrance [27].

5. Conclusion

The plants shows medicinal properties and they are antidepressant. Antioxidant and antimicrobial status of *Acorus calamus* and *Myristica fragrans* was done in this study. The Study of antioxidant activity was done with all extracts of both plants by DPPH method. *Acorus calamus* Showed antioxidant property. For the study of antimicrobial properties, Disc diffusion method was used to study. The acetone extract of *Acorus calamus* and *Myristica fragrans* showed antimicrobial property. Analysis of tannin from acetone extract of *Acorus calamus* was studied by TLC, Determination of total tannin, Bate smith, Hagerman Butler method. According to the current Study, The study indicates that *Acorus calamus* and *Myristica fragrans* shows less antioxidant and antimicrobial property. As discussed earlier, phenolics such as tannin was reported. The study indicates the presence of tannin. More studies on these plants help to study the uses of these plants.

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