

Review Article

## Chemical Constituents and Biological Activities of Promising Aromatic Plant Nagarmotha (*Cyperus scariosus* R.Br.): A Review

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*Cyperus scariosus* R.Br. (Nagarmotha) is pestiferous perennial, delicate slender sedge found wildly in various parts of the country, especially in damp or marshy areas and collected wildly for extraction of its essential oil using steam distillation. The rhizomes of *C. scariosus* possess pleasant aromatic odour, the essential oil is used as anti-inflammatory, anti-microbial and anti-fungal agent and it is also used as one of the ingredients in several formulations for the Ayurvedic systems of medicine. Phytochemical studies have shown that the major chemical components of this herb are polyphenol, flavonol, glycoside, alkaloid, saponins, sesquiterpenoids and essential oil. Rhizome of the plant is used in fever, arthritis, diuretic, nervine tonic, treatment of diarrhea and dysentery, leprosy, bronchitis, amenorrhea and blood disorders. The fruits of the plant are used as carminative, diuretic tonic and have stomachic. The details of plant and its essential oil, chemical constituents and biological activities have been described in this review.

**Key Words:** Aromatic Plants; Essential Oil; Chemical Constituents; Biological Activities; Nagarmotha; *Cyperus scariosus*; Cyprene

### Introduction

*Cyperaceae* family includes approximately 3000 species out of which about 220 species are identified as weeds. The genus *Cyperus* comprises more than fifty-two species that grow in damp or marshy places in India. The nut sedges originate from tropical and subtropical areas. The plant produces rhizomes, tubers, basal bulbs and fibrous roots below ground and rosettes of leaves, escapes and umbels above ground. *Cyperus scariosus* R.Br. also known as Nut grass, Nagarmotha and Nagarmustak is a delicate, slender sedge of this genus. The rhizomes of Nagarmotha occur 3-4 cm deep in soil and dug out from the soil (Fig. 1). Rhizomes and stolons have a number of wiry roots. Stolons are 10-20 cm long with a number of rhizomes crowded together. The

rhizomes are bluntly conical and vary in size and thickness. They are initially white and fleshy with scaly leaves and then become fibrous, wiry and very dark brown with age (Fig. 2).

*C. scariosus* is an angiosperm belonging to family *Cyperaceae*, wildly present in and around rivers, waterfall and other damp places. The plant requires sun and moist conditions, though it grows in sandy soil (one of the old Chinese names for it was shacao, meaning sand weed), as well as in loamy moist fields and particularly in Pacific Islands (where its leaves are used in weaving) as well as along coastal regions. *Cyperus* grows rapidly and fills the soil with its tangle of roots and rhizomes. *C. scariosus* is basically perennial, its height is approximately 45-75 cm. Leaves are sharp, pointed and 0.3-0.85 cm

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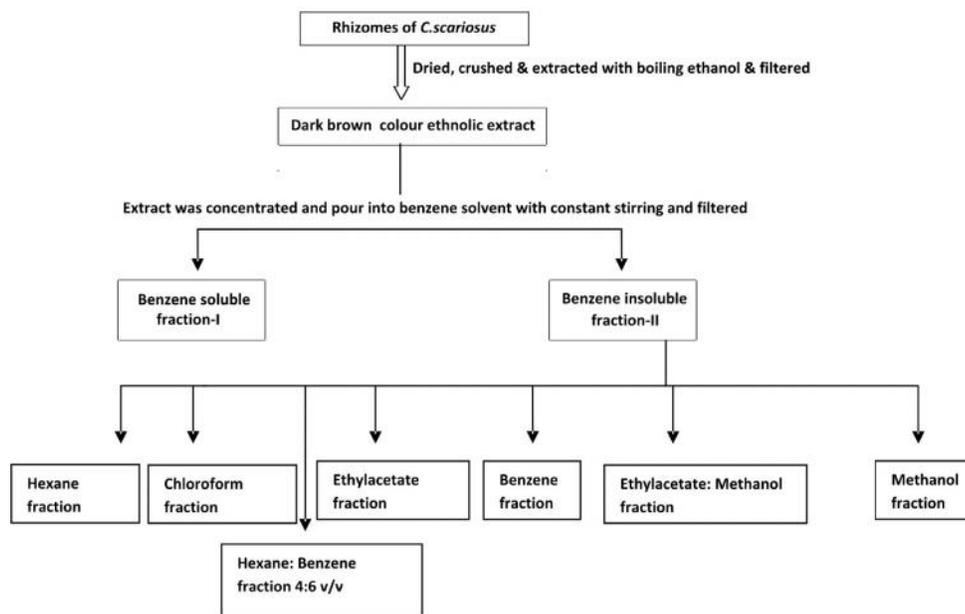


Fig. 1: Harvested Rhizomes of Nagarmotha ready for distillation

wide: flowers are 5-17.5 cm long. In *C. scariosus*, flowering is seen in July and fruits are formed in December. Only a few scattered reports are available in the literature on germination and nursery practices of *C. scariosus*.

### Habitat

There is no consistency in the literature citing the habitat of *C. scariosus*. Some authors have described nut grass as an annual and others as biennial or pluri-annual. It is not clear whether the plant behaves differently due to climatic conditions or varying genotypes. It is especially found in forest and swamp area but there is little data that specifies its bioavailability. Muni Ram *et al.* (1997) reported different yield and quality of essential oil of *C. scariosus* grown under assured water supply regions of central U.P. It has been found to grow in marshy land, river and canal banks and low or damp type areas of different parts of the country. It has also been observed that this plant thrives well under upland conditions where water resource are not a limiting factor.

### Distribution

*Cyperus scariosus* is widely distributed in India,

especially in Chhattisgarh, Bihar, Orissa, West Bengal and Uttar Pradesh. Nagarmotha is also found in South Africa, China and Pacific Islands. It is considered troublesome in 92 countries and adversely affects more than 30 crops. The plant is well known for its uses in the Indian System of Medicine (ISM) for a variety of purpose. Chopra *et al.* (1986) reported that *C. scariosus* grows in damp places in U.P., Bengal and southern part of India. It is also present in Chattarpur and Katni districts in Madhya Pradesh and adjoining areas of U.P particularly Jhansi and Banda, as reported by Jain (1989).

*Cyperus* is known as xiangfu/xiangfuzi in Chinese. The term xiang means fragrant and usually is applied to strong and pleasant fragrances, such as those occurring in culinary spices, perfumes and incenses. Anon (1950) mention that *Cyperus* is referred to as nut grass or nut sedge, the nut is the rhizome (or tuber), which forms rounded or elongated balls along a tangle of their roots. *Cyperus scariosus* is delicate slender sedge found in damp places reported by Chopra *et al.* (1992). The roots and tubers of *C. scariosus* are important ingredients of several Ayurvedic preparations. The commercial oil of *Cyperus* is known as cyperiol or oil of cyperiol, which is obtained from the rhizomes of *C. scariosus* by

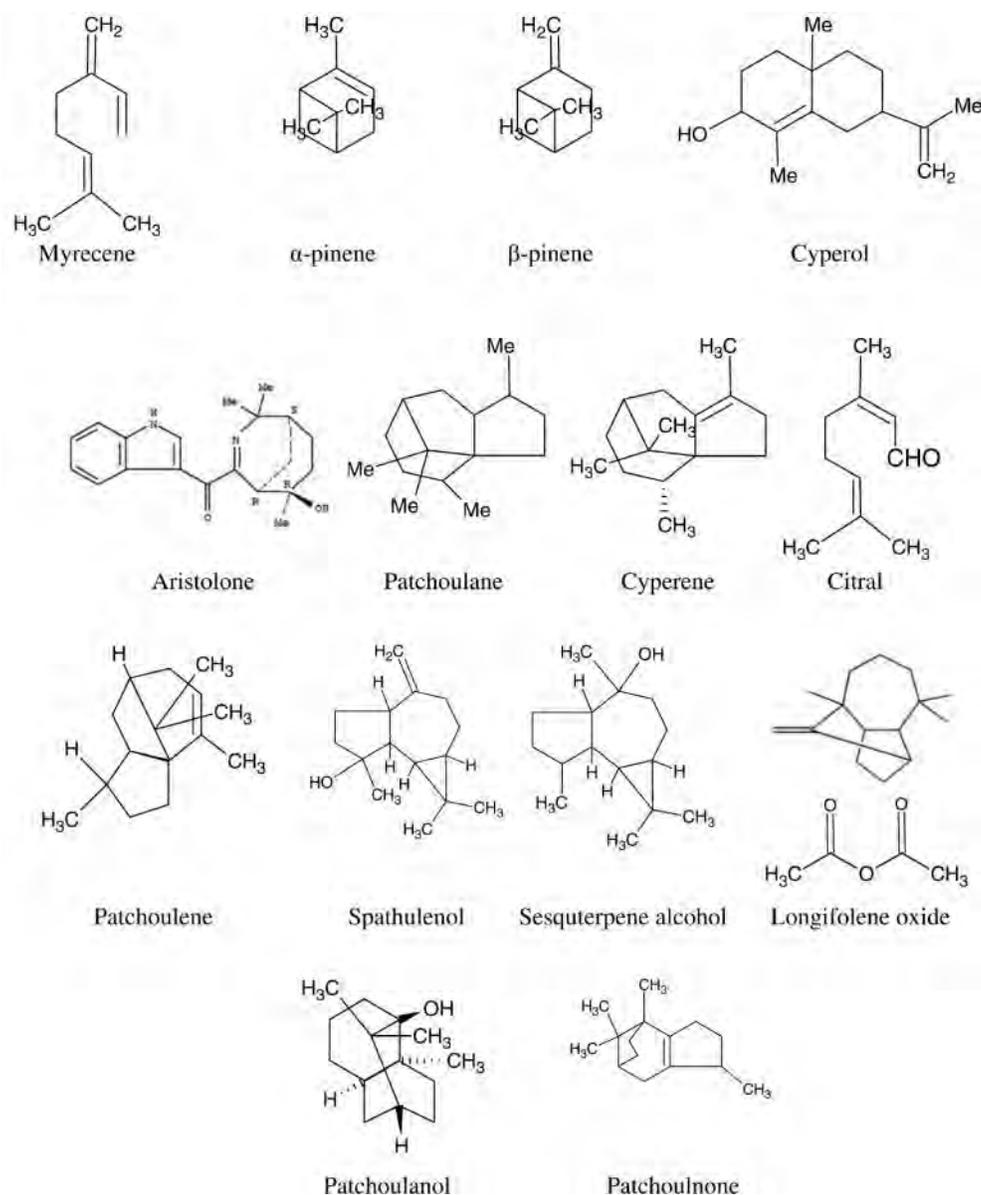


Fig. 2: Mature plants of Nagarmotha at FFDC, Kannauj

hydro-distillation. Besides distillation, rhizomes are used as such in 'dhoops', 'havansagri', hina, hair oils and also as medicines. The leaves of plant are used for making mats. Some people partially burn it to remove the rootlets occurring on it. The main market of *C. scariosus* rhizomes is in Mau-Ranipur of Jhansi district (U.P), from where the perfumers get the supplies of the material for their processing. The rhizome of *Cyperus* is rich in protein and ash content.

### Extraction of Oil and Purification of Compounds from the Rhizome

The prevalent method for extraction of essential oil from the rhizome of *C. scariosus* is steam distillation. In this process, essential oil is obtained by passing steam through dried crushed rhizome. In this process the steam is generated by a boiler, which is connected by a pipe to the still or tank. The pressure of steam is controlled according to the nature of plant material

which is being distilled. The tank/still, condenser and receiver-cum-separator are made-up of stainless steel. Basically, the steam distillation process is used for hardy and costly plant materials. First of all it is dried, crushed and extracted with boiling ethanol then filtered and dark brown color ethanol extract is found. After concentration of extract, it is macerated in benzene through which two fractions are obtained. First fraction has benzene soluble constituents while second fraction has benzene insoluble constituents. Light brown constituent of second fraction is loaded in sintered column and eluted with different organic solvent in increasing order of polarity. The chloroform is used for elution and chloroform soluble constituents are separated through this fraction. Ethyl acetate, benzene, methanol are further used for elution. Through each fraction of organic solvent we get many compounds in purified form, as reported by Sahu *et al.* (2010).

#### Chemical Composition of Essential Oil of *C. scariosus*

*Cyperus scariosus* has number of major and minor chemical constituents, many of which may show the pharmacological activities, but the main active components appear to be the sesquiterpenes. These are aromatic, spicy tasting molecules among main sesquiterpenes identified in *Cyperus* rhizomes. These are cyperone, selinene, cyperene, cyperotundone, patchulene, sugeonol, kobusone and isokobusone, pinene (monoterpene) derivatives of sesquiterpenes such as cyperol, isocyperol and cyperone. These active constituents are found in the volatile oil of *Cyperus* rhizomes, which make up only about 0.5-1% of the dried rhizome. Prolonged cooking of the herb will cause loss of some portion of these constituents. Their main pharmacological actions may be antispasmodic and analgesic effects.

Alcohol, aldehyde, ester, terpenes, hydrocarbon, ketone and miscellaneous compounds were tentatively identified by several workers. Garg *et al.* (1989) reported cyperene (15.74%), Yusuf *et al.* (1994) reported pinene (8.84%), camphene (11.40%), trans pinocarveol (10.53%), myrtenol (3.54%),

verbenone (2.25), cyperene (2.47%)  $\beta$ -selinene (2.75%), spathulenol (5.99%), cryophyllene oxide (7.15%), myrtenal (6.41%), limonene (3.22%), copaene 1 hycloprop (a) naphthalene,  $1\alpha,2,3,3\alpha$  (7.6%), zicrone (10.02%). However, Vazefafaj-Hury (2003) reported cyperene (24.42%), alfa-copaene (3.22%),  $\alpha$ -selinene (2.22%),  $\alpha$ -selinene (1.33%), iso-patchoulene (2.29%), corymbolone (11.91%). Uppal *et al.*, (1984) identified and reported several constituents in *C. scariosus* while, Chowdhury and Gupta (1998) reported the presence of pinene (14.18%), patchoulane (9.27%), cyperene (17.17%), longifolene oxide (24.61%), citral (6.14%), aristolene (7.29%) as major constituents. While studying the chemical constituents of essential oils from the rhizome, various authors have reported approximately 100 compounds. The detail of chemical composition (major and minor) is given in (Table 1).

#### Variation on Quality Due to Change in Harvesting Period

The harvested rhizome is used for extraction of essential oil after crushing or making powder. Muni Ram *et al.* (1997) reported the changes in quality of essential oil of *C. scariosus* due to different periods of harvesting from different places mainly Mandla and Raipur. The *C. scariosus* rhizome harvested in the month of January from Mandla had cis-pinocarveol -11.5%, sesquiterpene hydrocarbon-5.6%, iso-pathoul-3-ene -1.2%, cyperene -14.3%, rotundene -7.6%, curcumene -1.2%, sesquiterpene alcohol -7.2%, agarol-1%, rotundone-2.5%, corymbolone -2.2% while rhizome harvested from Raipur had cyperol cis-pinocarveol-5.5%, sesquiterpene hydrocarbon-8.6%, iso-pactchoul-3-ene-1.7%, cyperene-16.3%, rotundene-8.6%, curcumene-3%, sesquiterpene alcohol-14.4%, agarol-0.9%, rotundone-4.4% and corymbolone-4.5%. *C. scariosus* harvested in month of February from Mandla had cis-pinocarveol-9%, sesquiterpene hydrocarbon -7.7%, iso-pactchoul-3-ene-1.1%, cyperene -12.8%, rotundene -8.2%, curcumene-2.7%, sesquiterpene alcohol -1.2%, agarol-0.6%, rotundone-3.5%, corymbolone -3% while Raipur harvested rhizome had cyperol cis-pinocarveol-4.4%,

**Table 1 : Comparative chemical composition of *C. scariosus* reported by different researchers****Table 1a: List of Terpene present in Nagarmotha (*C. scariosus*)**

SNo.	Chemical constituent	Oladipupo A <i>et al.</i> (2009)		A.K. Panday <i>et al.</i> (2002)	Kedar Vaze <i>et al.</i>	Chowdhury <i>et al.</i> (2005)	Garg <i>et al.</i> (1989)
		Sample A	Sample B	Sample of Chindwara (M.P.)	Nagarmotha oil	Sample of Bangladesh	Nagarmotha oil
1.	$\alpha$ -pinene	3	10.8	0.75	0.13	8.84	—
2.	camphene	—	1.5	—	—	11.26	—
3.	$\beta$ -pinene	5.3	11.3	1.77	0.22	—	—
4.	limonene	1.7	0.6	—	—	—	—
5.	$\alpha$ -copaene	2	5.7	0.3	0.03	—	—
6.	cyperene	—	0.5	—	3.22	—	—
7.	$\beta$ -caryophyllene	1.6	2.6	13.91	24.42	2.47	15.75
8.	$\alpha$ -gurjunene	0.8	0.6	1.21	—	—	—
9.	allo-aromadendrene	—	0.3	3.53	—	—	—
10.	eudesma-2,4,11-triene	1.2	0.8	0.87	—	—	—
11.	$\beta$ -selinene	2.1	—	—	—	—	—
12.	$\alpha$ -selinene	5.1	4.6	2.48	2.22	2.75	3.00
13.	2-cyclopropylthiophene	2.7	6.6	2.48	1.33	—	—
14.	oxo-a-ylangene	—	2.5	—	—	—	—
15.	Oxygenated monoterpenes	—	1.9	—	—	—	—
16.	Oxygenated Sesquiterpenes	16	15.5	—	—	—	—
17.	germacrene D	45.1	27.9	—	—	—	—
18.	Copaene	—	—	2.11	—	—	—
19.	Rotundene	—	—	5.52	—	—	—
20.	Longifolene	—	—	5.76	—	—	—
21.	(+) $\delta$ -cadinene	—	—	2.69	—	—	—
22.	$\alpha$ -pinene	—	—	2.11	1.34	—	—
23.	eudesma-2,4,11-diene	—	—	4.55	—	—	—
24.	Guaiajulene	—	—	3.21	—	—	—
25.	Valencene	—	—	—	1.44	—	—
26.	ar-himachalene	—	—	—	1.9	—	—
27.	Copaene	—	—	—	—	7.6	—
28.	isopatchoul-3-ene	—	—	—	—	—	2.34
29.	Rotundenes	—	—	—	—	—	4.75
30.	iso-patchoul-4(5)-en-3-one	—	—	—	—	—	16.5

**Table 1b: List of oxides Present in *Cyperus scariosus* reported by different researches**

SNo.	Chemical constituent	Oladipupo A <i>et al.</i> (2009)		A.K. Panday <i>et al.</i> (2002)	Chowdhury <i>et al.</i> (2005)
		Sample A	Sample B	Sample of Chindwara (M.P.)	Sample of Bangladesh
1.	$\alpha$ -gurjunenepoxide [2]	—	—	—	0.96
2.	aromadendrene epoxide	—	2.7	—	—
3.	caryophyllene oxide	5.4	2.6	12.45	7.15
4.	humulene epoxide II	2.7	1.6	—	—

**Table 1c: List of Aldehydes present in *C. scariosus* reported by different researchers**

SNo.	Chemical constituent	Oladipupo <i>et al.</i> (2009)		Chowdhury <i>et al.</i> (2005)
		Sample A	Sample B	Sample of Bangladesh
1	Myrtenal	—	—	6.41
2	(2R,5E)-caryophyll-5-en-12-al	1	—	—
3	Cuminaldehyde	trace	0.1	—

**Table 1d: List of Alcohol present in *C. scariosus* reported by different researchers**

SNo.	Chemical constituent	Oladipupo <i>et al.</i> (2009)		Panday <i>et al.</i> (2002)	Kedar Vaze <i>et al.</i>	Garg <i>et al.</i> (1989)
		Sample A	Sample B	Sample of Chindwara (M.P)	Nagarmotha oil	Nagarmotha oil
1	Fenchol	—	—	0.2	—	—
2	trans-pinocarveol	4	4	4	7.24	10.53
3	camphene hydrate	—	—	0.4	—	—
4	p-mentha1-5diene-8-ol	0.4	0.4	—	—	0.97
5	Borneol	—	—	0.3	—	—
6	terpinen-4-ol	0.9	0.9	1	—	—
7	Myrtenol	7.9	7.9	7.1	—	3.54
8	trans-carveol	trace	Trace	0.4	—	—
9	spathulenol	0.3	0.3	—	0.62	5.99
10	Globulol	—	—	0.9	—	—
11	Patchenol	3.9	3.9	0.9	—	—
12	caryophylla-3,8(13)-dien-5- $\beta$ -ol	4.2	4.2	2.4	—	—
13	vulgarol B	3.8	3.8	1.8	—	—
14	caryophylla-3,8(13)-dien-5-a-ol	2.1	2.1	—	—	—

sesquiterpene hydrocarbon-8.1%, iso-pactchoul-3-ene-1.3% , cyperene -15.8%, rotundene -9 %, curcumene-3%, sesquiterpene alcohol -12%, agarol-1.5%, rotundone-3.1% and crymblone -3%. The rhizome harvested in month of March from Mandla had cis-pinacorveol-3%, sesquiterpene hydrocarbon-8.4%, iso-pactchoul-3-ene- 0.3%, cyperene-12.3%, rotundene -10.7 %, curcumene-2.6%, sesquiterpene alcohol -11.7%, agarol-0%, rotundone-2.7%, crymblone -3.1% while in Raipur rhizome, cyperiol cis-pinacorveol-4.3%, sesquiterpene hydrocarbon-7.7%, iso-pactchoul-3-ene-0%, cyperene -11.2%, rotundene -8.2 %, curcumene-3.3%, sesquiterpene alcohol -11.6%, agarol-1.7%, rotundone-0%,crymblone -2.5% were reported (Table 2).

### Phytochemical Investigations

Phytochemical studies have shown that major chemical components of this herb are polyphenol, flavonol, glycoside, alkaloid, saponins, sesquiterpenoids and essential oil, cardiac glycosides. The main chemical constituents of *Cyperus scariosus* are pinene, patchoulane, cyperenolongifolene oxide, citral, aristolene, isopatchoulene, cyperenone, cyperenol, patchoulene, scariodonecyperotundone, rotundone, cyperene-I (a tricyclicsesquiterpene), cyperene-II (a bicyclicsesquiterpene hydrocarbon), patchoulene, mustakone, cyperotandone (cyperenone),  $\beta$ -selinene, cyperone, copadieneepoxyguanine, rotundone, eugenol, cyperol, isocyperol,  $\alpha$ -rotundol,  $\beta$ -rutonol, kobusone,

**Table 2: Variation in chemical constituents of essential oil of *C. scariosus* due to changes in harvesting time and place Ram *et al.* (1996)**

Compound	Jan		Feb		March		April	
	Mandla	Raipur	Mandla	Raipur	Mandla	Raipur	Mandla	Raipur
cis-pinocarveol	11.5	5.5	9.0	4.4	30.	4.3	2.8	3.0
Sesquiterpene hydrocarbons	5.6	8.6	7.4	8.1	8.4	7.7	5.6	6.2
Iso-patchoul-3-ene	1.2	1.7	1.1	1.3	0.3	0	1	0
Cyperene	14.3	16.3	12.8	15.8	12.3	11.2	9.4	9.8
Rotundene	7.6	8.6	8.2	9	10.7	8.2	8	10.5
Curcumene	1.2	3	2.7	3	2.6	3.3	1	0
Sesquiterpene alcohol	7.2	14.4	1.2	12	11.2	11.6	10.6	8.1
Agarol	1	0.9	0.6	1.5	0	1.7	0	0
Rotundone	2.5	4.4	3.5	3.1	2.7	0	2.5	2.2
Corymbolone	2.2	4.5	3	3	3.1	2.5	2	0

isokubusone,  $\beta$ -selinene,  $\alpha$ -cyperone caryophyllene-6,7-oxide, caryophyllene-6-one and caryophyllene. The sesquiterpene, ketone and alcohols constitute about half of the essential oil. Garg *et al.* (1988) identified volatile constituents of the essential oil of *Cyperus scariosus* tubers. Three new sesquiterpenoids have been isolated and their structures elucidated by spectroscopic method and chemically transformed. For identification, four different spectroscopic methods were used a. is co-injection with authentic material, b. is mass spectroscopy (MS), c. is  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, d. is Infrared spectroscopy (IR).

The details of chemical composition were examined by GC and GC-MS that identified the major components of the compounds. Chowdhary and Gupta (1998) investigated the constituents present in essential oil and found major hydrocarbons: myrcene (0.5%),  $\alpha$ -pinene (1.2%),  $\beta$ -pinene (14.18%), patchoulone (9.27%), cyperene (17.17%),  $\beta$ -selinene (4.26%), isopatchoulene (2.7%), longifolene oxide (24.61%), alcohol: spathulenol (4.85%), patchoulanol (1.8%), cyperol (2.0%), sesquiterpene alcohol [M]<sup>+220</sup>, Aldehyde: citral (6.14%), Ketone: aristolone (7.29%), cyperolone (0.05%) were reported along with many minor constituents (Table 3).

**Table 3: Chemical components of the essential oil identified through various methods by Garg *et al.* (1989)**

S.No.	Constituents	Identified by*	% in total oil
1	cyperene(4)	a,b,c,d	15.75
2	Rotundene	b,c	4.75
3	Rotundone	B	5.10
4	iso-patchoul-4(5)-en-3-one	b,c,d	16.50
5	Patchoulone	b,c,d	7.60
6	sesquiterpenediketone[M] <sup>232</sup>	B	3.46
7	Corymbolone	B	3.15

\*a=co-injection with authentic material, b=mass spectroscopy (MS), c= $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, d=infrared spectroscopy (IR)

Gupta *et al.* (1972) reported anti-inflammatory activity of essential oil isolated from *Cyperus scariosus*. Lahariya *et al.* (1979) studied *in-vitro* antimicrobial activity of cyperiol. Dikshit *et al.* (1984) reported antifungal action of some essential oil against animal pathogens. Joshi *et al.* (2000) reported indigenous knowledge and uses of medicinal plants by local communities of Kali Gandaki Watershed Area, Nepal. The plant is mentioned in the ancient Indian ayurvedic text *Charaka Samhita* (ca.100AD). Modern ayurvedic medicines use plants for treating fevers, digestive system disorders, dysmenorrhea and other maladies. In the traditional Chinese medicine, it is considered the primary regulating herb. Traditionally in Arabs, the use of roasted tubers treated the wounds, bruises, carbuncles, etc. Chopra *et al.* (1992) reported that rhizomes of *Cyperus scariosus* yields 0.077-0.080% essential oil with a pleasant odour and used as stomatic, diaphoretic, astringent; and useful in diarrhea, gonnorrhoea and syphilitic affections. While, Yusuf *et al.* (1994) reported that besides essential oil, the rhizomes are also used in dhoops, hair oils and in medicines.

### Pharmacological Effect of Major Isolates from Nagarmotha Oil

#### *$\alpha$ -pinene and $\beta$ -pinene*

Renbaum-Wolff Lindsay *et al.* (2013) using two unique techniques, namely a “bead-mobility” technique and a “poke-flow” technique, in conjunction with simulations of fluid flow, the viscosity of the water-soluble component of SOM produced by  $\alpha$ -pinene ozonolysis is quantified for 20 to 50  $\mu$ m particles at 293-295 K. The viscosity is comparable to that of honey at 90% relative humidity (RH), similar to that of peanut butter at 70% RH, and at least as viscous as bitumen at 30% RH, implying that the studied SOM ranges from liquid to semisolid or solid across the range of atmospheric RH. Rivas da Silva *et al.* (2012) reported antimicrobial activities of the isomers and enantiomers of pinene were evaluated against bacterial and fungal cells. The agar diffusion test showed that only the positive enantiomers of the  $\alpha$ - and  $\beta$ -isomers of pinene were active confirming that

the positive enantiomers exhibited microbicidal activity against all fungi and bacteria tested with MICs ranging from 117-4,150  $\mu$ g/mL. However, no antimicrobial activity was detected with the negative enantiomers up to 20 mg/mL. Time-kill curves showed that (+)- $\alpha$ -pinene and (+)- $\beta$ -pinene were highly toxic to *Candida albicans*, killing 100% of inoculum within 60 min. By contrast, the bactericidal effect occurred after 6 h in methicillin-resistant *Staphylococcus aureus* (MRSA). In combination with commercial antimicrobials, ciprofloxacin plus (+)- $\alpha$ -pinene or (+)- $\beta$ -pinene presented synergistic activity against MRSA whereas an indifferent effect against all fungi was detected when amphotericin B was combined with the positive enantiomers of pinene. Wijayati Nanik *et al.* (2012) reported that homogenous acid was used as catalyst for the hydration of  $\alpha$ -pinene using water as hydroxyl donor, which is soluble in aqueous and organic solvents.  $\alpha$ -pinene is the main component of most turpentine oils. In order to obtain more valuable products,  $\alpha$ -pinene in the turpentine can be hydrated in dilute mineral acid solutions to produce  $\alpha$ -terpineol, which can be used as perfume, repellent of insect, antifungal and disinfectant. The highest selectivity of terpineol was 87.56% with a conversion of 88.21% after 30 min of reaction at 70°C. Rachwalik Rafal *et al.* (2007) reported that Isomerization of  $\alpha$ -pinene was performed on a series of dealuminatedferrierite (FER)-type zeolites in liquid phase at 363 K using a batch reactor. The course of zeolite dealumination was followed in detail using <sup>29</sup>Si, <sup>27</sup>Al, <sup>1</sup>H MAS NMR, XRD, FTIR and sorption of nitrogen. The ammonium form of FER was dealuminated with aqueous solutions of HCl. While retaining the crystallinity of the zeolite particles, the treatments removed up to 53% of the tetrahedrally coordinated aluminum atoms from the FER framework.

#### *D-limonene*

Toro-Arreola Del *et al.* (2005) reported that D-limonene and its metabolites have been shown to exert chemo preventive and chemotherapeutic activities against different tumors in animal models and clinical trials. However, it is unknown whether these compounds modulate the immune response in

tumor-bearing mice. We evaluated the survival of lymphoma-bearing mice fed with a diet with D-limonene. To assess the cell immune response, we sensitized and challenged BALB/c mice with 2,4-dinitrofluorobenzene (DNFB) and evaluated the T-cell subpopulations by flow cytometry. We also examined phagocytosis, microbicidal activity and chemotactic function in peritoneal macrophages. In order to know the role of D-limonene and its metabolites, macrophage NO production and lymphocyte proliferation studies were performed *in-vitro* with D-limonene, perillal acid and perillyl alcohol. The results showed that D-limonene increased the survival of lymphoma-bearing mice, delayed hypersensitivity reaction to DNFB, phagocytosis and microbicidal activity. *In-vitro* studies indicate that D-limonene increased NO production in peritoneal macrophages obtained from tumor-bearing mice. Thangaiyan Rabi *et al.* reported that D-limonene and docetaxel in combination significantly enhanced the cytotoxicity to DU-145 cells than PZ-HPV-7 cells. Exposure of DU-145 cells to a combined D-limonene and docetaxel resulted in higher ROS generation, depletion of GSH, accompanied by increased caspase activity than docetaxel alone. It also triggered a series of effects involving cytochrome *c*, cleavages of caspase-9, 3 and poly (ADP-ribose) polymerase, and a shift in Bad:Bcl-xL ratio in favor of apoptosis.

Apoptotic effect was significantly blocked on pretreatment with *N*-acetylcystein, indicating that antitumor effect is initiated by ROS generation, and caspase cascades contribute to the cell death the first time, that D-limonene enhanced the antitumor effect of docetaxel against prostate cancer cells without being toxic to normal prostate epithelial cells. The combined beneficial effect could be through the modulation of proteins involved in mitochondrial pathway of apoptosis. D-limonene could be used as a potent non-toxic agent to improve the treatment outcome of hormone-refractory prostate cancer with docetaxel. Yoon *et al.* (2010) studied the pharmacological and biological effects of D-limonene on the production of pro-inflammatory cytokines and inflammatory mediators in macrophages. The results indicate that D-limonene is an effective inhibitor of

lipopolysaccharide (LPS)-induced NO and prostaglandin E (2) production in RAW 264.7 cells. These inhibitory effects of D-limonene included dose-dependent decreases in the expression of iNOS and COX-2 proteins. To evaluate the inhibitory effects of D-limonene on other cytokines, we also measured TNF-alpha, IL-1beta, and IL-6 levels in the cell supernatants of LPS-stimulated RAW 264.7 macrophages by enzyme-linked immunosorbent assay. In these assays, D-limonene decreased the expression of TNF-alpha, IL-1beta, and IL-6 in a dose-dependent manner. To assess the suitability of D-limonene for cosmetic applications, we also performed 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assays on HaCaT keratinocytes. D-limonene did not display any cytotoxicity in these assays. From these results, we suggest that D-limonene may be considered a potential anti-inflammatory candidate. John Whysner *et al.* (1996) reported that D-limonene produces tumors only in the kidneys of male rats in association with hyaline-droplet nephropathy, which is due to the accumulation of the rat-specific, low molecular weight protein  $\alpha_{2u}$ -globulin in the P2 segment cells of renal proximal tubules. Human urine does not contain  $\alpha_{2u}$ -globulin and, compared with the male rat, much less protein and almost no low molecular weight protein. Genotoxicity tests for D-limonene are negative, and the mechanism of tumorigenesis involves tumor promotion and enhanced cell proliferation. There is no risk of cancer for humans from D-limonene, since the binding of D-limonene to  $\alpha_{2u}$ -globulin would not occur. However, Ramakrishnan Murali *et al.* (2010) studied to evaluate the protective effects of D-limonene on the levels of lipid peroxidation by-products and antioxidant defense systems in the plasma and tissues of normal and streptozotocin (STZ)-induced diabetes rats. The experimental diabetes was induced in rats by a single dose of STZ (40 mg/kg i.p.) injection, and treatment with D-limonene was continued for 45 days. After the treatment period, oxidative stress parameters such as lipid peroxidation by-products; enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase; non-enzymic antioxidants including reduced

glutathione, Vitamins C and E were measured in the plasma and tissues of experimental rats. An increase in the levels of lipid peroxidation by-products and significant decrease in antioxidant enzymes were observed in untreated diabetic rats. Administration of D-limonene to diabetic rats for 45 days caused a significant reduction in the levels of lipid peroxidation by-products and an increase in the activities of antioxidant enzymes, when compared with the untreated diabetic group. There was no significant difference in normal treated groups, when compared with normal rats.

### ***Germacrene D***

Bulow *et al.* (2000) reported that Germacrene D is considered as a precursor of many sesquiterpene hydrocarbons. The acid catalyzed the photochemically and thermally induced rearrangement processes of Germacrene D isolated from several *Solidago* species, which contain both enantiomers of Germacrene D. Rostelien *et al.* (2000) reported single receptor neurons on the antennae of the tobacco budworm moth, *Heliothis virescens* were screened for their sensitivities to naturally produced plant volatiles by the use of gas chromatography linked to electrophysiological recordings from single cells (GC-SCR). Plant volatiles, collected by aeration of host and non-host plants, were tested on each receptor neuron via parallel GC-columns. Thus, simultaneous recordings of the gas chromatogram and the neuron responses to each component were obtained. One type of receptor neuron, appearing in 80% of all experiments, responded with high sensitivity and selectivity to one particular component, present in host as well as non-host mixtures. The component, identified as a sesquiterpene hydrocarbon by linked gas chromatography-mass spectrometry was isolated from a sesquiterpene fraction of cubebe oil and identified by NMR as Germacrene D. The purified compound was then re-tested via gas chromatography on the same receptor neuron type, verifying the identification. A weaker response to another sesquiterpene hydrocarbon was also recorded.

### ***Myrtenal***

*Hepatocellular carcinoma* is a primary malignancy of the hepatocytes, which rapidly leads to death in short periods. The aim of this study was to investigate the possible therapeutic efficiency of myrtenal against diethylnitrosamine-induced experimental hepatocarcinogenesis by analyzing the key enzymes of carbohydrate metabolism, lysosomal and mitochondrial TCA cycle enzymes, and also the possible role of tumor suppressor protein p53, and scanning electron microscopic studies. The results revealed that myrtenal significantly ameliorated the altered enzymes of carbohydrate metabolism, lysosomal and mitochondrial enzymes, and interestingly the tumor suppressor protein p53 was found to be significantly accumulated in myrtenal-treated animals, which inevitably confirms that myrtenal has a prominent role in preventing the liver cancer.

### **Conclusions**

The scientific studies on essential oils, biological models and its clinical studies have proved that the essential oils of *C. scariosus* have various uses in fragrance, flavor, pharmaceuticals and aromatherapy and are effective in the treatment of various diseases and inflammatory conditions. This study explores one of the promising and effective medicinal sources from these wild plants. As we know, *Cyperus* is a wild plant present in nature and there sufficient systematic information is not available about them due to lack of research. There is lot of scope to carry out research works on various issues related to these plants.

Different species of *Cyperus* such as *C. scariosus* and *C. rotundus* when applied to different extraction methods give different types of oil; absolute and resinoid which can be utilized as such in fragrances, flavour, pharmaceutical and aromatherapy or could be utilized to make different isolates. Presently, the essential oil of Nagarmotha is extracted from the rhizome collected only from wild sources so the availability of oil have limited uses in varied products. However, intensive screening is required to get best quality of germplasm for promoting its cultivation, processing and

commercialization because it has tremendous pharmacological uses with perfumery value. There are a number of activities shown by different isolates of Nagarmotha. However, in some cases two or more isolates work together for pharmaceutical values. On the basis of above observation it is clear that the other isolate may also show some positive results. *Cyperus* family has so many pharmacological and biological activities and there is a lot of possibility to find out lead compounds with marked specific properties as in other known plants and hence may

prove to be a great discovery in the field of pharmaceutical industry.

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