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### Pande Jyoti

Phytochemical, Pharmacological and Microbiological laboratory, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat-360005, India

### Padalia Hemali

Phytochemical, Pharmacological and Microbiological laboratory, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat-360005, India

### Rokad Nilam

Phytochemical, Pharmacological and Microbiological laboratory, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat-360005, India

### Chanda Sumitra

Phytochemical, Pharmacological and Microbiological laboratory, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat-360005, India

### Correspondence:

#### Chanda Sumitra

Phytochemical, Pharmacological and Microbiological laboratory, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat-360005, India

Email: svchanda[at]gmail.com

## *Cyperus conglomeratus* (Cyperaceae) a halophyte from Gujarat: Physicochemical, Phytochemical and Pharmacognostic studies

Pande Jyoti, Padalia Hemali, Rokad Nilam, Chanda Sumitra\*

### ABSTRACT

The aim of the present work was evaluation of physicochemical, phytochemical and pharmacognostic parameters of *Cyperus conglomeratus* an halophyte from Gujarat. The plant belonging to the family Cyperaceae commonly known as Sedge family. The leaf and stem was evaluated macroscopically and microscopically. Preliminary qualitative phytochemical analysis, physicochemical analysis which included loss on drying, ash values and extractive values and fluorescence analysis were done in accordance with WHO guidelines for identification and standardization of *C. conglomeratus*. The morphological studies exhibited the organoleptic and surface characteristics of whole plant like the leaf was simple, alternate, elongated lanceolate, entire margin, acute apex, parallel venation. The microscopic and powder microscopic study showed the presence of various characteristics like parenchyma cells, spiral vessels, annular vessels, parallel venation, gramineous stomata, conjoint collateral vascular bundle, etc. The phytochemical analysis revealed the plant to be rich in steroids and triterpenes. The extractive value was maximum in methanol solvent indicating presence of more polar compounds. The fluorescence analysis under visible light & under UV light by treatment with different chemical reagents showed characteristic colour changes. The standards laid down will be helpful to ensure the efficacy, safety and purity of medicinal halophytic plant *C. conglomeratus*. The parameters established in this study are useful for the preparation of a monograph of this plant.

**Keywords:** *Cyperus conglomeratus*, Cyperaceae, physicochemical analysis, phytochemical analysis, microscopic study, powder microscopy, fluorescence analysis, leaf, stem.

### INTRODUCTION

Cyperaceae also known as Sedge family belongs to monocotyledonous graminoid flowering plants. They superficially resemble grasses and rushes, however their distinguishing characters are stems with triangular cross-sections and leaves that are spirally arranged in three ranks [1]. It includes grass like plants which grow mostly in marshy places. It comprises about 4000 species with 90 genera. Many *Cyperus* species are used as food and medicines. The tubers of *Cyperus esculentus* are edible and used as spermatogenic, aphrodisiac, galactagogue, emollient, digestive, tonic, diuretic and promotes menstruation [2]. *Cyperus rotundus* is a weed plant with many biological and pharmacological activities to its credit. In folk medicine, it is used to treat many diseases like dyspepsia, fever, skin diseases, dysmenorrhoea, cancer, diarrhoea, renal and vesical calculi, etc [3]. The essential oil cypriol from *Cyperus scariosus* is used in medicine and perfumes [4]. Many other species in the genus *Cyperus* like *C. alopecuroides*, *C. rotundus*, *C. articulatus* and *C. maculatus* also produce aromatic essential oil. The essential oil cypriol is highly in demand in perfumery because of its unique woody, ambery, warm, spicy, balsamic character with feature of cedarwood and vetiver. *Cyperus conglomeratus* is a monocotyledon and perennial plant with loutish rhizomes growing up to 12-15 cm long and 0.2-0.3 cm width [5]. It is indigenous to India, but is now found in tropical, subtropical and temperate regions [6]. It is a common perennial weed with slender and sheathing leaf base. The stems grow to about 12-15 cm tall and the leaves are linear, dark green and grooved on the upper surface. Inflorescences are small, with 2-4 bracts, consisting of tiny flowers with a red-brown husk. The nut is three-angled, oblong-ovate and yellow in colour.

Marine halophytes are specialized group of plants adopted for high saline conditions which include mangroves, seaweeds, sea grass and blue green algae. They are distributed from coastal region to inland deserts and have been traditionally used for medicinal and nutritional purposes. Halophytes are able to tolerate harsh saline and arid conditions because of various phytoconstituents they synthesize which make them fit and adaptable. This is achieved by developing adaptive responses including the biosynthesis of different primary and secondary metabolites such as saponins, flavonoids, triterpenes,

steroids, cardiac glycosides, anthocyanins, phlobatanins, phenols, alkaloids, coumarins, etc which display several biological activities. For eg. *Mesembryanthemum crystallinum* and *Carpobrotus edulis* show antioxidant and antibacterial activity [7], *Limonium densiflorum* show anti-inflammatory activity [8], *Reaumuria vermiculata* show anticancer activity [9], *Limonium tetragonum* show antiobesity activity [10], etc.

The standardization and quality control measures of the herbal medicines is necessary to assure the quality of the drugs, like allopathic medicine. Standardization parameters will also help in checking and preventing substitution and adulteration, which is nothing but mixing or substituting the original drug material with other spurious, substandard, defective, spoiled, useless other parts of the same or different plants [11] which can occur in herbal medicine especially in plants which are short supplied or highly in demand. The simplest and easiest method is pharmacognostic studies which does not require any sophisticated modern equipments. According to WHO, standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects such as selection and handling of crude material, safety, efficacy and stability assessment [12]. Pharmacognostic study involves organoleptic evaluation, macroscopic and microscopic characterization, powder studies, phytochemical analysis, physicochemical analysis, fluorescence analysis, etc.

Pharmacognostic and biological studies of some halophytes from Gujarat has already been reported from our laboratory. For eg. *Ipomea pes-caprae* L., [13]; *Limonium stocksii* (Boiss) Kuntze [14]; *Trianthema portulacastrum* L., [15]; *Suaeda nigra* L., [16]; *Salvadora oleoides* Decne and *Salvadora persica* L. [17], etc. Pharmacognostic studies of plants from different habitats is also reported in literature. For eg. algae [18], halophytes [13], succulent plant [19], terrestrial plants [20, 21], etc. In the present study, pharmacognostic, physicochemical, phytochemical analysis of *Cyperus conglomerates*, an important halophyte from Gujarat has been attempted.

## MATERIAL AND METHODS

### Collection of plant material

*Cyperus conglomeratus* Rottb. was collected in the month of August, 2017 from Porbandar, Gujarat, India. The plant was thoroughly washed under tap water to remove soil and other debris and shade dried. The dried plant material was homogenized to fine powder and stored in closed container for further studies.

### Pharmacognostic study

#### Macroscopic studies

Pharmacognostic study was done by organoleptic evaluation. The morphological features of different parts of the plant were observed under magnifying lens. Macroscopic characters were studied using

standard methods [22]. Photographs at different magnifications were taken by using digital camera.

### Microscopic studies

Microscopic studies were carried out by preparing thin sections of leaf and stem of *C. conglomeratus* plant. They were then washed with water, stained with different reagents like safranin and fast green; they were mounted in glycerine for observation and confirm its lignifications (10x, 40x). The powder microscopic study of entire plant dry powder was done and specific diagnostic characteristic features were recorded according to Tyler *et al.*, [23].

### Physicochemical analysis

The physicochemical analysis involved measurement of parameters like moisture content in other words, loss on drying, various ash values like total ash, acid-insoluble ash, water-soluble ash, carbonated ash, nitrated ash and sulphated ash. The extractive values were measured in solvents of different polarity. The solvents used were water (AQ), methanol (ME), ethyl acetate (EA), toluene (TO), and petroleum ether (PE). The details of the procedure followed is as described by Rakholiya *et al.*, [24].

### Phytochemical analysis

The qualitative phytochemical analysis of crude powder and different solvent extracts of whole plant powder of *C. conglomeratus* was carried out to identify different phytoconstituents [25]. The phytoconstituents analysed were alkaloids, flavonoids, phenols, saponins, tannins, cardiac glycosides, steroids, phlobatanins, triterpenes, anthocyanins, etc. The presence of specific phytochemicals indicated is indicated with (+) sign and the absence of phytochemicals is indicated with (-) sign. The procedure followed for different phytochemical analysis is as described by Rokad *et al.*, [13].

### Fluorescence analysis

The fluorescence analysis of leaf and stem of *C. Conglomerates* carried out following the method of Kokaski *et al.* [26]. The procedure followed is as described by Pandavadra and Chanda [27]. The colours observed by application of different reagents in visible light, short (254 nm) and long (365nm) ultra violet radiations was observed and recorded.

## RESULTS

### Organoleptic and macroscopic characteristics

Organoleptic and macroscopic characteristics are given in Table 1. The leaf was simple, alternate, elongated lanceolate, margin entire, apex acute, venation parallel, with characteristic odour and taste. The average leaf size was 12-15 cm in length and 0.2-0.5 cm in width.

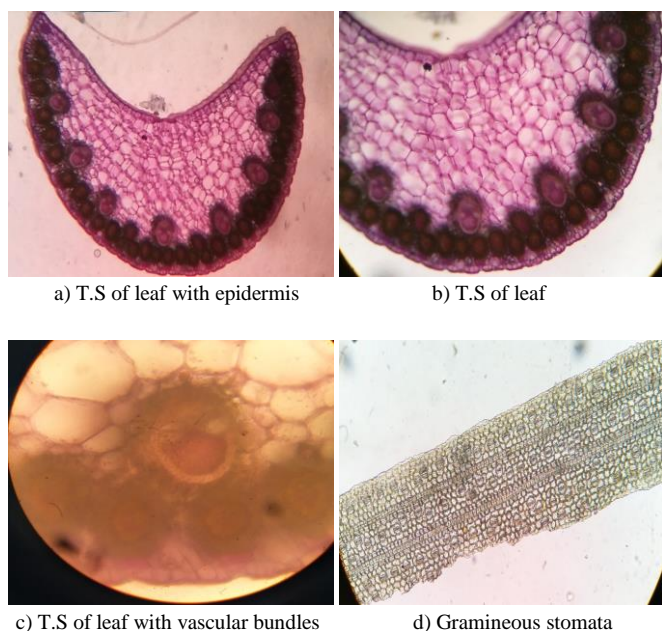
**Table 1:** Organoleptic features of *Cyperus conglomeratus*

Characters	Observation
Part	Leaf
Arrangement	Alternate
Size	12-15 cm long, 0.2-0.5 cm wide
Shape	Elongated lanceolate
Color	Green
Odour	Characteristic
Taste	Characteristic
Appearance	Smooth
Margin	Entire
Apex	Acute
Base	Symmetrical
Petiole	Sessile (without petiole)
Texture	Smooth
Veination	Parallel
Outer surface	Glabrous

**Microscopic characteristics**

**Leaf**

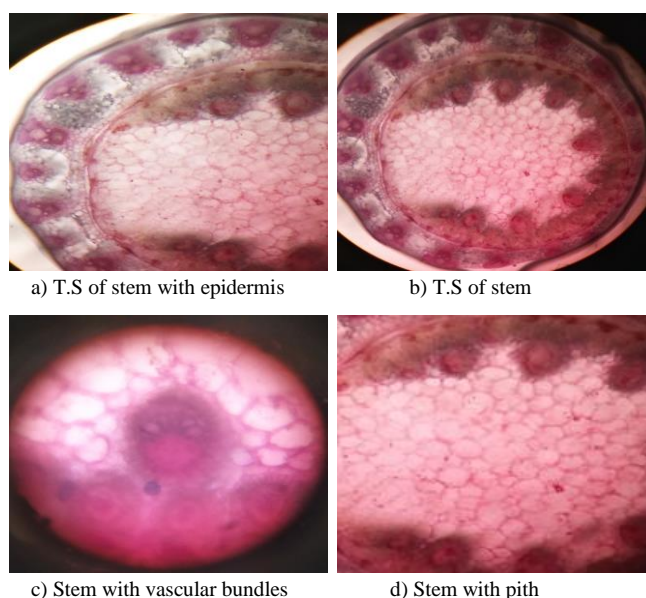
The transverse section of *C. conglomeratus* leaf is shown in Fig. 1. The leaf lamina was dorsiventral in nature. The upper epidermis and lower epidermis was single layered (Fig. 1a). The auxiliary elongated lower epidermal cells with slight wavy walls were embedded with gramineous stomata at certain intervals. The transversely cut leaf lamina showed unequal sized parenchymatous cells (Fig. 1b). The groups of fibres and vascular bundles were embedded in parenchymatous cells on the dorsal surface, the vascular bundles were collateral, conjoint and closed type (Fig. 1c). The gramineous stomata were present on the lower epidermis. The guard cells were dumb-bell shaped; gramineous stomata were surrounded by small subsidiary wavy cells, whereas the guard cells were comparatively larger in size (Fig. 1d).



**Figure 1:** Microscopic study of leaf of *C. conglomeratus*

**Stem**

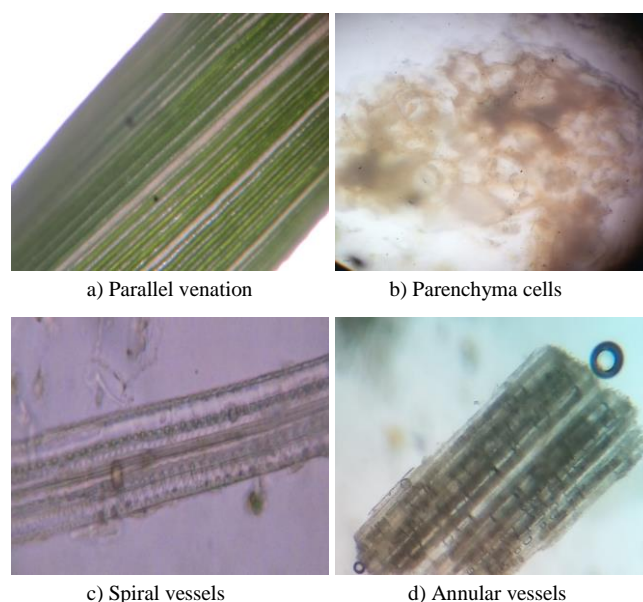
The transverse section of *C. conglomeratus* stem is shown in Fig 2. The epidermis was single layered and was surrounded by thick cuticle (Fig. 2a). The epidermal hairs or trichomes were absent and the ground tissue was not differentiated into cortex and pith (Fig. 2b). The vascular bundles were scattered in sclerenchymatous cells and each vascular bundle was oval shaped, conjoint collateral and closed type. Xylem was endarch, phloem was represented only by companion cells, sieve tubes and little phloem fibres (Fig. 2c). Pith was very large and consisted of thin walled sclerenchymatous cells (Fig. 2d).



**Figure 2:** Microscopic study of stem of *C. conglomeratus*

**Powder microscopy of plant**

The crude powder of *C. conglomeratus* plant was dark green in colour, fine, odour was characteristic and taste was slight acrid. The powder microscopy characteristics are shown in Fig. 3. The specific characteristics determined from the powder study under microscopic investigation showed parallel venation, parenchyma cells, spiral vessels, annular vessels, gramineous stomata, etc (Fig. 3).



**Figure 3:** Powder study of *C. conglomeratus*



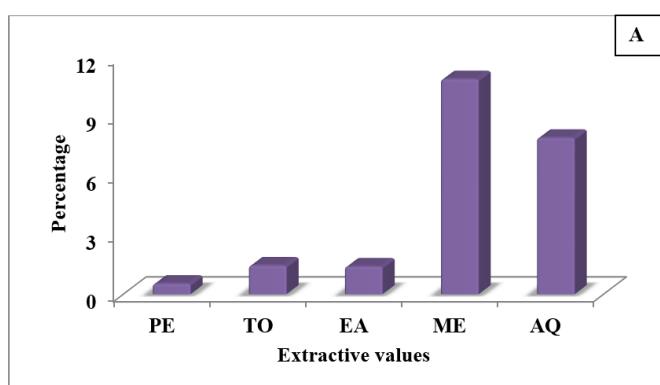
**Physicochemical analysis**

**Moisture content (loss on drying)**

The method generally used to determine moisture content is to heat the drug in an oven at 100 – 105 °C till constant weight. The loss on drying of dry powder of plant was 15.5 % which is as described for other plants in literature.

**Extractive values**

This parameter determines the amount of particular active chemical constituent present in a particular solvent from a known amount of plant material. It is generally denoted in percentage values. The extractive values of plant powder is given in Fig. 4a. The maximum soluble extractive value was in methanol solvent (10.9 %) and minimum in petroleum ether solvent (0.54 %). The water soluble extractive value was 7.93 % (Fig. 4a).

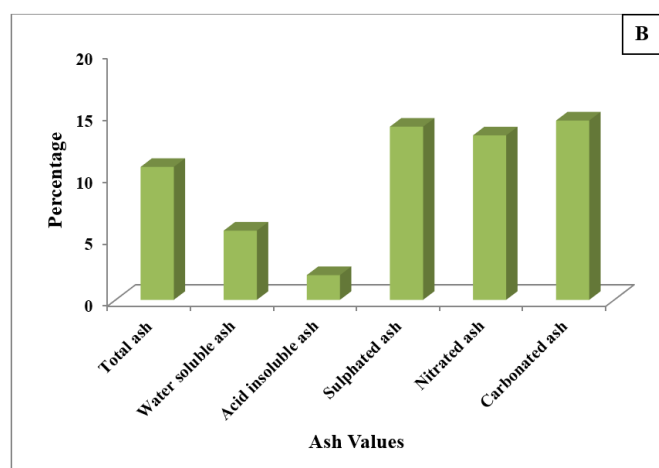


**Figure 4A:** Extractive values of different solvent extracts of *C. conglomerates*

**Ash values**

Evaluation of ash values determines the quality and purity of the crude drug under study. The total ash was 10.75 %, while water soluble ash and acid insoluble ash was 5.6 % and 2 % respectively.

The sulphated, nitrated and carbonated ash of plant powder was 14 %, 13.3 %, 14.5 % respectively (Fig. 4b).



**Figure 4B:** Ash values of crude powder of *C. conglomerates*

**Phytochemical analysis**

The qualitative phytochemical screening of crude powder and different solvent extracts of *C. conglomeratus* whole plant is given in Table 2. In the crude powder of the whole plant, steroids were present in maximum amount; while alkaloids, tannins, saponins, triterpenes and leucoanthocyanins were present in trace amount. The phytoconstituents in various solvent extracts varied. In PE solvent extract, flavonoids, steroids, triterpenes, coumarins and quinones were present in trace amount (Table 2). In TO solvent extract, steroids and triterpenes were present in moderate amount while alkaloids, flavonoids and coumarins were present in trace amount. In EA solvent extract, triterpenes were present in maximum amount followed by steroids and coumarins; alkaloids and flavonoids were present in trace amount. In ME solvent extract, tannins were present in moderate amount while saponins and triterpenes were present in trace amount. In AQ solvent extract, alkaloids and saponins were present in moderate amount while triterpenes were present in trace amount.

**Table 2:** Qualitative phytochemical analysis of *Cyperus conglomeratus*

No.	Phytochemicals	Whole plant powder	PE	TO	EA	ME	AQ
1	Alkaloids						
	(1)Mayer’s reagent	+	-	-	-	-	-
	(2)Dragondroff’s reagent	+	-	-	-	-	++
	(3)Wagner’s reagent	+	-	+	+	-	-
2	Flavonoids	-	+	+	+	-	-
3	Tannins	+	-	-	-	++	-
4	Phlobatanins	-	-	-	-	-	-
5	Saponins	+	-	-	-	+	++
6	Steroids	+++	+	++	++	-	-
7	Cardiac glycosides	-	-	-	-	-	-
8	Triterpenes	+	+	++	+++	+	+
9	Anthocyanins	-	-	-	-	-	-
10	Phenols	-	-	-	-	-	-
11	Coumarins	-	+	+	++	-	-
12	Leucoanthocyanins	+	-	-	-	-	-
13	Quinones	-	+	-	-	-	-

(+++ more amount, (++) moderate amount, (+) less amount, (-) absent

### Fluorescence analysis

The fluorescence characters of powdered drugs of plants play a very important role in the determination of quality and purity of the drug material. In the present study, dried powder of the plant was treated with a number of different reagents which showed characteristic

fluorescence under visible light and UV light at short wave length (254 nm) and long wavelength (365 nm) (Table 3). The plant powder showed different colours at both the wavelengths. The colours visualized were brown, dark brown, black, light black and green. In some reagents the colour in visible light was brown which turned to black in UV light or it turned from brown to green (Table 3).

**Table 3:** Fluorescence analysis of *Cyperus conglomeratus*

No.	Treatment	Under visible light	Under UV light short wave length(254 nm)	Under UV light long wave length(365 nm)
1	1N NaOH (Aq)	Brown	Black	Black
2	1N NaOH (alco)	Brown	Black	Brown
3	Ammonia	Brown	Black	Green
4	Petroleum ether	Brown	Black	Brown
5	50% HCl	Brown	Black	Brown
6	50% H <sub>2</sub> SO <sub>4</sub>	Brown	Black	Brown
7	Ethyl acetate	Brown	Green	Brown
8	Ethyl alcohol	Brown	Black	Brown
9	Methanol	Brown	Black	Brown
10	50% KOH	Brown	Black	Brown
11	50% HNO <sub>3</sub>	Red	Black	Black
12	Acetic acid	Brown	Light Black	Brown
13	Iodine in water (1%)	Green	Black	Dark Brown
14	FeCl <sub>3</sub>	Brown	Black	Light Black

### DISCUSSION

One of the impediments in the acceptance of the herbal products worldwide is the lack of standard quality control profiles. Standardization parameters are important and necessary in the beginning of any research and in acceptance of the herbal drugs by people in general. It will help in checking and preventing adulteration and substitution. Standardization and quality control measures taken at all steps from collection, production and manufacturing of the drugs, will lead to maintenance of efficacy and reproducible quality of the drug. Quality control ensures that the plant material is not contaminated and that the final product is of consistent high standard.

In the present study, pharmacognostic study was done by evaluating macroscopic and microscopic studies, physicochemical studies, phytochemical studies and fluorescence analysis. Organoleptic and macroscopic evaluation is a primary evaluation based on the study of morphological profile of the plant. The macroscopic evaluation of *C. conglomerates* showed that the plant was green in colour, simple, leaf was alternate, shape was elongated lanceolate, entire margin, acute apex, parallel venation. The microscopic evaluation showed leaf lamina was dorsiventral, gramineous stomata, parenchymatous cells and collateral close vascular bundles. The salient diagnostic characteristics of stem were thick cuticle layer, sclerenchymatous cells. vascular bundle collateral conjoint close, xylem was endarch and ground tissue was not differentiated into cortex and pith. The powder microscopy evaluation showed parallel venation, parenchyma cells, spiral vessels, annular vessels, gramineous stomata, etc. The organoleptic, macro and microscopic characteristics and powder study revealed the specific characters of *C. conglomerates* which are the diagnostic characters of this plant. It will help to identify the plant

when intact and when in powder form. These parameters will help to correctly identify the plant and prevent it from being adulterated.

The moisture content or loss on drying of the drug is an important parameter since it will give an idea about the drying process of the plant whether drying is complete or partial or insufficient. If the plant is not properly dried, it will lead to the growth of bacteria and fungi and also decomposition of the active principle occurs. It will decrease the shelf life of the plant drug [28]. In *C. conglomeratus* loss on drying was 15.5 %. This indicates that drying process was efficient and within limits. It is not high enough for microbial contamination. Loss on drying for leaf of *Brunfelsia Americana* was 15.2 % [29], for leaf of *Persicaria odorata* was 14.49 % [30].

Ash values of a drug gives an idea about the inorganic composition and other impurities present along with the drug. The ash values are constant for a given drug and therefore it is one of the diagnostic parameter of the drug. In certain drugs, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug sometime possess a character that will raise the ash value. Ash involves oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. In the present study, the ash values ranged from 2 % to 11 %. Total ash value was 10.75 %, while acid insoluble ash was 2 %. These values indicate the amount of organic and inorganic material present in the plant sample. The acid insoluble ash normally contains silica and earthy material and indicates contamination. In the present work, it was very negligible hence it can be stated that the plant material is free from contamination. The ash values are in accordance with those reported

for other plants. For eg. total ash value was 12 %, water soluble ash was 3%, acid insoluble ash was 1.5% for *Syzygium cumini* Linn leaf [31], total ash value was 12 -14 %, water soluble ash was 5-7 %, acid insoluble ash was 4 – 5% for *Vitex leucoxylo* Linn leaf [32].

Extractive values give an idea about the chemical constituents of crude drugs and also help in estimation of definite constituents soluble in a particular solvent. The extractive values of *C. conglomerates* in organic solvents ranged from 0.54% to 10.9 %. Maximum extractive value was in methanol and minimum in petroleum ether and water soluble extractive value was 7.93%. The results indicated presence of more polar compounds than non polar compounds in *C. conglomerates*.

Phytochemical analysis conducted on the plant extracts reveal the presence of constituents which are known to exhibit medicinal as well as physiological activities [33]. Analysis of the plant extracts revealed the presence of various type of phytochemicals in different amount. The crude powder of *C. conglomerates* was rich in steroids; while its solvent extract EA, was rich in triterpenes. The other phytoconstituents were either present in trace amount or absent in other solvent extracts.

The therapeutic efficacy of plants is not part specific. Each part rather every part of the plant shows some pharmacological activity. Hence pharmacognostic study of all parts is done and reported for various organs of the plant. For e.g. leaf, stem and root [34], leaf [35], stem [36], bark [37], flower [38], fruit [39], seed [40], root [41], aerial parts [42], etc.

## CONCLUSION

To our knowledge, this is the first attempt on pharmacognostic study of *Cyperus conglomerates* especially from Gujarat coast. Hence this study is of quite importance and the parameters studied here will be a good measure to maintain the quality assurance of this plant and prevent the crude drug from being adulterated. It will help in maintaining the authenticity, efficacy and repeatability of the drug characteristics.

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