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IN VITRO PHARMACOGNOSTICAL EVALUATION OF *CYPERUS KYLLINGA* ROOT

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ABSTRACT

Background: *Cyperus kyllinga* is an important and rare medicinal plant in India. In this study we have taken only the root part of this plant. The main background of studies focused on macroscopic, microscopic, physicochemical and phytochemical analysis. **Objective:** To evaluate in vitro pharmacognostical study of *Cyperus kyllinga* root. **Materials and Methods:** The Pharmacognostical parameters were carried out by complete botanical evaluation which includes macroscopic, microscopic, physicochemical and phytochemical analysis. **Results:** The results shows different organoleptic characters, also shows different microscopic characters like the pith is larger, the vascular bundles varies from 6-8, intercellular space is absent in between endodermis, this shows that it is a monocot root. Also shown starch grains, xylem, phloem, endodermis, sclerenchyma, epidermis, cortex, pith, pericycle, lignified xylem vessel and oil glands of the T.S. of *C. kyllinga* root. In the present study we also determine the different ash values, extractive values and Phytochemical identification like carbohydrate, reducing sugar, non reducing sugar, alkaloids, proteins, amino acid, cardiac glycoside, saponin glycoside, flavonoids and steroids. **Conclusion:** In indigenous system of medicine this studies are not done before. Here we want to establish the pharmacognostical standard serve as a reference piece and helps in the further identification and authentication of this taxon.

KEYWORDS: Microscopic study; Organoleptic studies; Fluorescence analysis; Ash value; Chemical tests.

INTRODUCTION

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.[1] India has several traditional medical systems, such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. The *materia medica* of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India. The ancient texts like Rig Veda (4500-1600 BC) and Atharva Veda mention the use of several plants as medicine. The books on ayurvedic medicine such as *Charaka Samhita* and *Susruta Samhita* refer to the use of more than 700 herbs.[2] According to the World Health Organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines.[3] Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain.[4] The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed.[5] Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bio prospecting of new plant-derived drugs.[6] The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine.[7] Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and

chemotherapeutics from these plants as well as from traditionally used herbal remedies.[8] The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them.[9,10] According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy.[11] The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges[12], therefore they are more prone to extinction.[13] Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes.[14] Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector.[15]

The long historical use of medicinal plants in many traditional medicinal practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy.[16] Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained. Today with the present surge of interest in the phyto-therapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of its study. It becomes extremely important to make an effort towards standardization of the plant material as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material.[17] So, the present study is undertaken to standardize *Cyperus kyllinga* pharmacognostically which will help in the correct identification of the drug.

***Synonyms or Vernacular names*[18]**

Synonyms: *Kyllinga nemoralis, Kyllinga monocephales*

Hindi: Nirbisi

Bengali: Nirbishaghas

Sanskrit: Svetnirvasi

Kingdom: Plantae

It consists of fresh leaves and dried roots of *Cyperus kyllinga*, belonging to family Cyperaceae.

***Geographical source*[18]:** Generally found in tropical parts of India. It is also found in China, Srilanka, Singapore, Malaysia, Phillipines and Indonesia.

***Cultivation and collection*[19,20]:** It grows on variety of soils,but the soil should be full of moisture is mostly found in grassy places & in shady areas. It is native to tropics of both hemisphere, particularly Asia. It is generally found in tropical region and wetty regions.

MATERIALS AND METHODS

The plant has been collected for pharmacognostical study from forest of Ichharia village (Sonamukhi range), Bankura, West Bengal, India. and authenticated by B.S.I.(CNH/71/2011/Tech.II/), Shibpur, Howrah, and West Bengal, India.

Morphological Characters: *Cyperus kyllinga* is 30cm in height. It is a smooth plant with creeping underground stem. Leaves are thin and long. Flowers are white. Roots are dark brown. Root have characteristic odour and having characteristic taste. Leaves are ¼ inch in diameter. Table 1 shows the different morphological characters of the *C. kyllinga* root.

Microscopic Characters: Figure 1, 2, 3 and 4 shows T.S. of *C. kyllinga* roots which consist different microscopic parts of the plant. The T.S. of the *CK* root was treated with different chemical reagents and this process is useful for detection of microscopic characteristics under ordinary day light by standard methods.[21] In Figure 1, 2, 3 and 4 staining has done by Phloroglucinol & Con.HCl (1:1), ruthenium red, N/50 iodine solution and Phloroglucinol & Con.HCl (1:1) respectively.[22,23]

Powdered microscopic study: Figure 5, 6 and 7 shows powder microscopy of *C. kyllinga* roots which consist different microscopic part of the powder form of the *C. kyllinga* root. The powdered form of the *Cyperus kyllinga* root was treated with different chemical reagents and

this process is useful for detection of powder microscopic characteristics under ordinary day light by standard methods.[21] To study the behaviour of powdered form of the *Cyperus kyllinga* root a pinch of powdered drug was treated with different chemical reagents like acetic acid, 1(N) NaOH, 1(N) HCl 1(N) HNO_3 , 5% Iodine , 5% Ferric chloride and 1(N) HNO_3 followed by ammonia solution and colors were observed shows in Table 2.[22,23]

***Fluorescence Analysis*[23,24]:** To study the fluorescence nature of powder, a pinch of powder after bleaching with 5% chloral hydrate was treated with different chemical reagents such as 1N HCl, 1N NaOH, 50% HNO_3, 50% H_2SO_4, methanol, acetic acid, ammonia, 5% iodine, ferric chloride and observed under UV light, shows in Table 3. Fluorescence analysis of different extracts of *Cyperus kyllinga* root by visible and ultra-violet (UV) radiations was performed by reported method observed in Table 4.

Physico-chemical analysis: Ash value determination (Total ash content, Acid insoluble ash and Water soluble ash) were calculated as per Indian pharmacopoeia.[25] Table 5 shows the value of total ash content, acid insoluble ash and water soluble ash. Figure 8 shows comparative study among the value of ash content. Successive extractive values were observed with solvents of chloroform, Alcohol and Aqueous.[26,27] Table 6 shows the different extractive value of the *C. kyllinga* root and Figure 9 shows the comparative study among the extractive value.

Phytochemical analysis: Different extracts (Pet. Ether extract, chloroform extrct, ethanol extrct and aqueous extract) are prepared for the study of preliminary phytochemical screening by using different reagents for identifying the presence of various phytoconstituents. The above phytoconstituents were tested as per the standard methods[28,29] shows in Table 7.

RESULTS

The present study establishes the different macroscopic (Table 1) and microscopic characteristics (Figure 1,2,3,4,) of *Cyperus kyllinga* root. Table 1 shows the result of different morphological characters of the *C. kyllingia* root. Figure 1 shows (T.S. of *C. kyllinga* root) Starch grains (A); Xylem (B); Phloem (C); Endodermis (D); Sclerenchyma (E) and Epidermis (F) when stained by Phlorogucinol and Con.HCl (1:1). Figure 2 shows T.S. of *C. kyllinga* roots with Staining reagent Ruthenium red. It shows Cortex (A_1) ; Phloem (B_1) ; Pith (C_1) ; Xylem (D_1) ; Pericycle (E_1) ; Epidermis (F_1). Figure 3 shows clusters of starch grains in the root of *C. kyllinga* when stained by N/50 iodine solution. Figure 4 shows endodermis and

lignified xylem vessels when stained by Phlorogucinol and Con.HCl (1:1). Figure 5, 6 and 7 shows powder microscopy of *C. kyllinga* roots like cork part, lignified xylem vessels and oil glands. Table 2 shows behaviour of the powdered form of *Cyperus kyllinga* root with different chemical reagents. This process is useful for detection of powder microscopic characteristics under ordinary day light by standard methods. The fluorescence analysis of powdered drug in day light, short UV and long UV were examined by reported method. The observations are given in Table 3. The Table 4 reveals the behaviour analysis of different solvent extract of *Cyperus kyllinga* root under visible light, short and long UV. In physicochemical screening Table 5 shows the value of total ash content, acid insoluble ash and water soluble ash. Figure 8 shows comparative study among the value of ash content. Table 6 shows the different extractive value of the *C. kyllinga* root and Figure 9 shows the comparative study among the extractive value. Table 7, shows the different phytochemical screening of the *Cyperus kyllinga* root extract (Pet.Ether extract, chloroform extrct, ethanol extrct and aqueous extract). From our study report it reveals that flavonoids, carbohydrate, reducing sugar, steroids, tannins, phenols, saponin, anthraquinone glucoside present in *Cyperus kyllinga* root.

Table. 1: Organoleptic characteristics of Cyperus kyllinga root part.

Organoleptic Parameters	***Cyperus kyllingia***
Colour (Root)	Deep brown
Taste (Root)	Bitter
Odour (Root)	Aromatic
Appearance (Root)	Coarse powder
Height (Plant)	30.0 cm
Height (Root)	09.0 cm
Diameter (root)	0.25 cm

Table. 2: Behavior of The powdered form of Cyperus kyllinga root with different chemical reagents.

Treatment	**Colour**
Powder	Brown
Powder + Acetic Acid	Deep brown
Powder + 1(N) NaOH	Dark brown
Powder + 1(N) HCl	Light brown
Powder + 1(N) HNO_3	Brownish red
Powder + 5% Iodine	Dark brown
Powder + 5% Ferric chloride	Yellowish brown
Powder + HNO3 + Ammonia solution	Light brown

Table. 3: Fluorescence analysis of Cyperus kyllinga root powder with different chemical reagents.

Treatment	Observation		
	Visible	Short UV (254 nm)	Long UV(366nm)
Powder	Brown color	Green color	Dark brown color
Powder + 1(N) NaOH	Dark brown color	Green color	Dark brown color
Powder + 1(N) HCl	Light brown color	Dark green color	Light brown color
Powder + Acetic Acid	Deep brown color	Greenish yellow color	Dark brown color
Powder + 50% HNO_3	Reddish brown color	Dark green color	Dark green color
Powder + 50% H_2SO_4	Brown color	Greenish brown color	Dark brown color
Powder + Ammonia	Light brown color	Greenish brown color	Dark brown color
Powder + 5% Iodine	Dark brown color	Dark green color	Dark brown color
Powder + Methanol	Light brown color	Brown color	Dark brown color
Powder + 5% Ferric chloride	Yellowish brown color	Yellowish green color	Dark brown color

Table. 4: Fluorescence analysis of different extracts of Cyperus kyllinga root by visible and ultra-violet (UV) radiations.

Extract	Observation		
	Visible	Short UV (254 nm)	Long UV(366nm)
Petroleum ether	Yellow brown	Brown	Black
Chloroform	Greenish brown	Greenish Brown	Black
Ethanol	Deep brown	Dark Brown	Dark Brown
Water	Dark Brown	Brown	Black

Table. 5: The value of the ash content in % w/w (mean ± SEM).

Parameters	(%w/w), (mean ± SEM)
Total ash content	10.5 ± 0.578
Acid insoluble ash	4.5 ± 0.305
Water soluble ash	3.5 ± 0.251

Table. 6: Extactive value of water, alcohol and chloroform in %w/w (mean ± SEM).

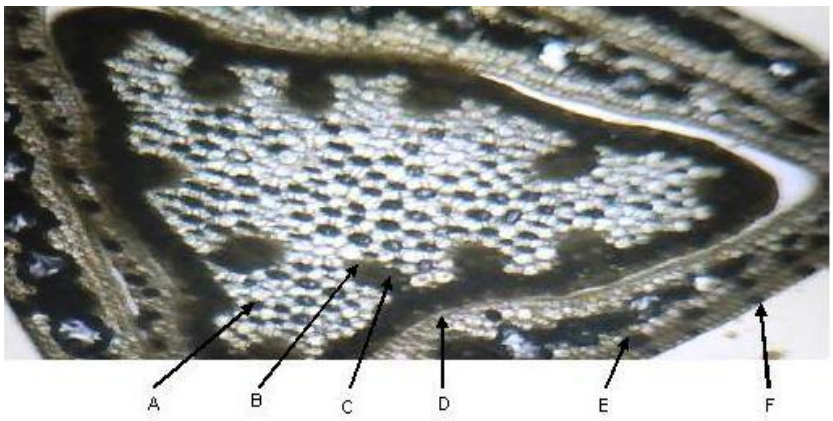
Solvent	(% w/w), (mean ± SEM)
Water	9.0 ± 0.360
Alcohol	4.4 ± 0.404
Chloroform	2.0 ± 0.2

Table. 7: Phytochemical screening of the C. kyllinga root.

Name of the test	Pet. Ether	Chloroform	Ethanol	Water
Test for Carbohydrate *Molish's Test*	-Ve	-Ve	+Ve	+Ve
Test for Reducing Sugar 1.*Fehling's Test* *2.Benedict's Test*	-Ve -Ve	-Ve -Ve	+Ve +Ve	+Ve +Ve
Test for Non Reducing Sugar	-Ve	-Ve	-Ve	-Ve
Test for Alkaloids *Wagner's test* *Dragendroff's test*	-Ve -Ve	-Ve -Ve	-Ve -Ve	-Ve -Ve
Test for Proteins *Biuret test* *Millon's test*	-Ve -Ve	-Ve -Ve	-Ve -Ve	-Ve -Ve
Test for Amino acid *Ninhydrin test*	-Ve	-Ve	-Ve	-Ve
Test for Cardiac glycoside *Baljet test* *Keller killani test*	-Ve -Ve	-Ve -Ve	-Ve +Ve	-Ve +Ve
Test for Anthraquinone glycoside *Borntrger's test*	-Ve	-Ve	+Ve	+Ve
Test for Saponin glycoside *Foam test*	-Ve	-Ve	+Ve	+Ve
Test for Flavonoids *Lead acetate test* *Ferric chloride test*	-Ve -Ve	-Ve -Ve	+Ve +Ve	+Ve +Ve
Test for Steroids	+Ve	+Ve	-Ve	-Ve
Test for Tannins and Phenolic compounds	-Ve	-Ve	+Ve	+Ve

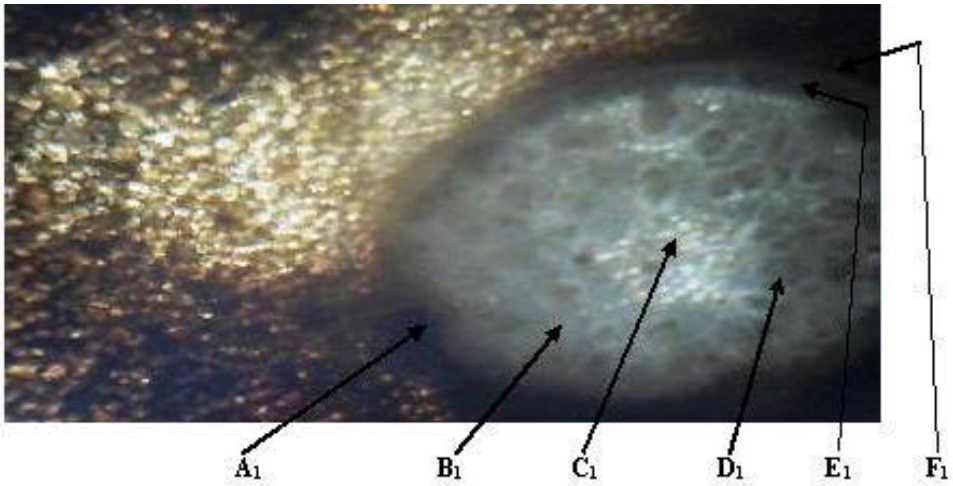
'+Ve' = Presence of the compound

'-Ve' = Absence of the compound



A. Starch Grains; B. Xylem; C. Phloem; D. Endodermis; E. Sclerenchyma; F. Epidermis.

Figure. 1: T.S. of C. kyllinga roots [Staining reagent : Phlorogucinol+Con.HCl (1:1)].



A_1. Cortex; B_1. Phloem; C_1. Pith; D_1. Xylem; E_1. Pericycle; F_1. Epidermis.

Figure. 2: Shows closer view of T.S. of C. kyllinga roots (Staining reagent: Ruthenium red).

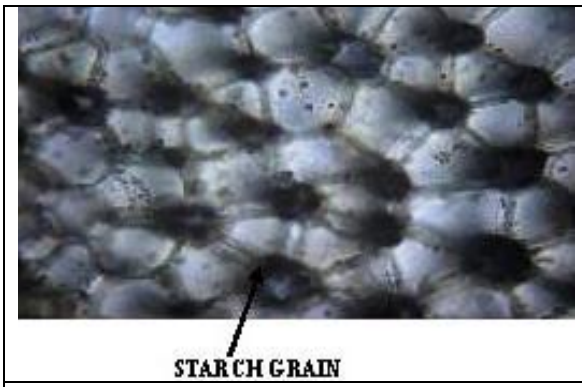


Figure. 3: Shows more closer view of clusters of starch - grains in the root of CK (Stained by N/50 iodine solution).

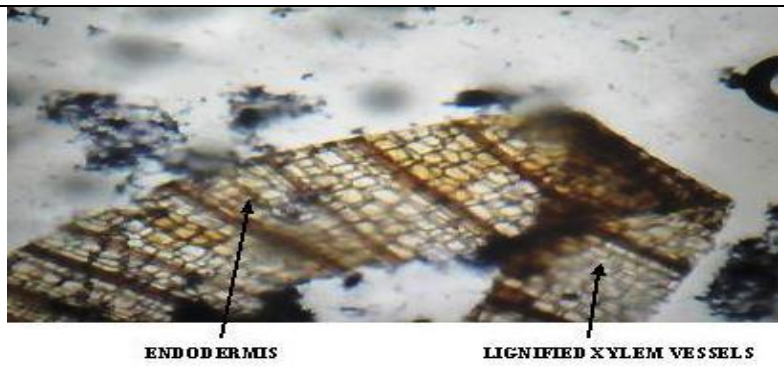


Figure. 4: Shows closer view of endodermis & lignified xylem vessels of CK root [Staining reagent: Phloroucinol+Con.HCl (1:1)].

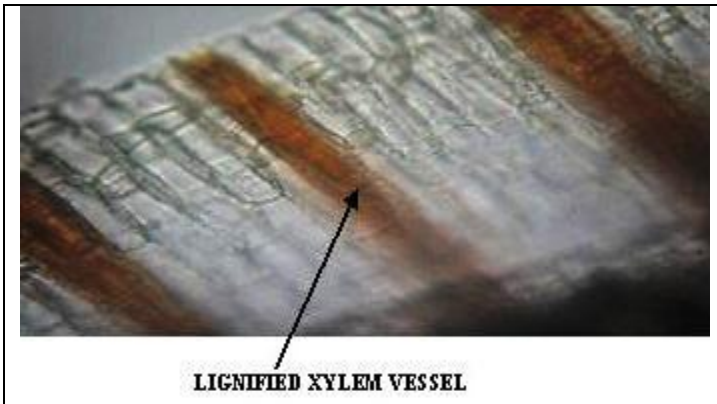


Figure. 5: Shows lignified xylem vessels of the powder microscopy of C. kyllinga roots [Staining reagent : Phloroucinol+HCl (1:1)].

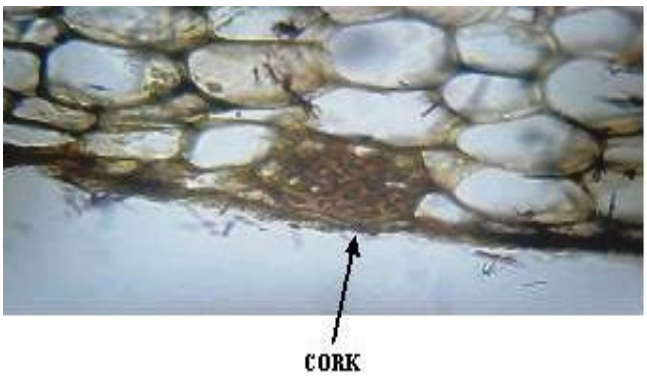


Figure. 6: Shows powder microscopy of C. kyllinga roots [Staining reagent : Phloroucinol+HCl (1:1)].

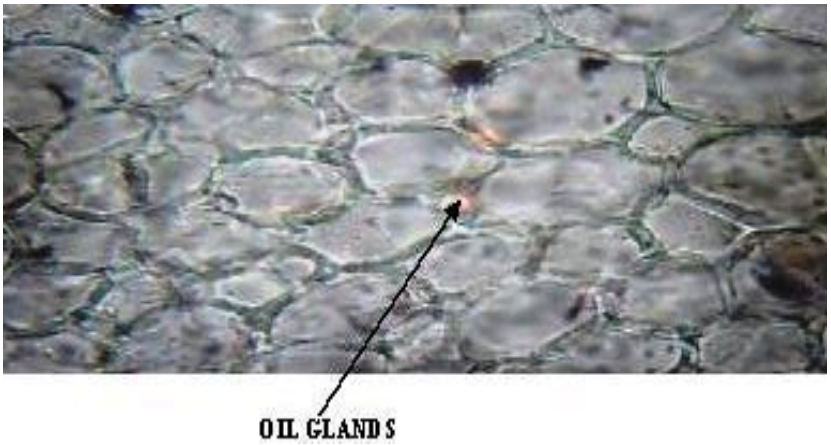


Figure. 7: Shows powder microscopy of C. kyllinga roots (Staining reagent: Sudan red III).

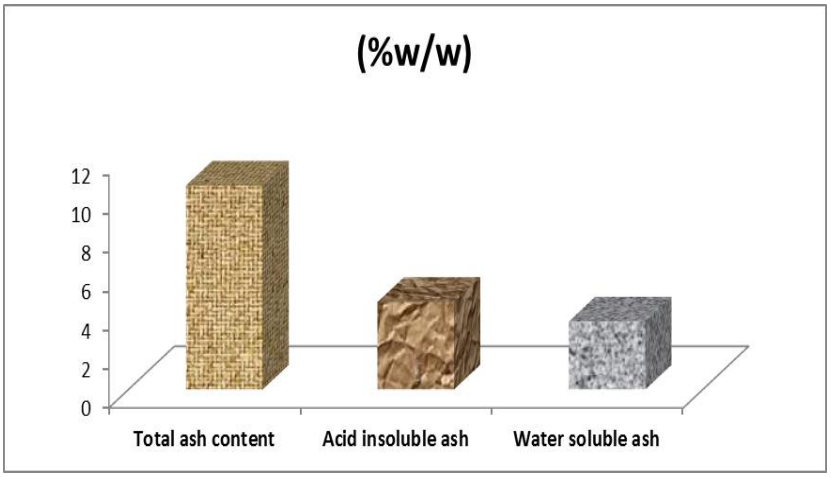


Figure. 8: Comparative study among the values of toatal ash content, acid soluble ash content and water soluble ash content.

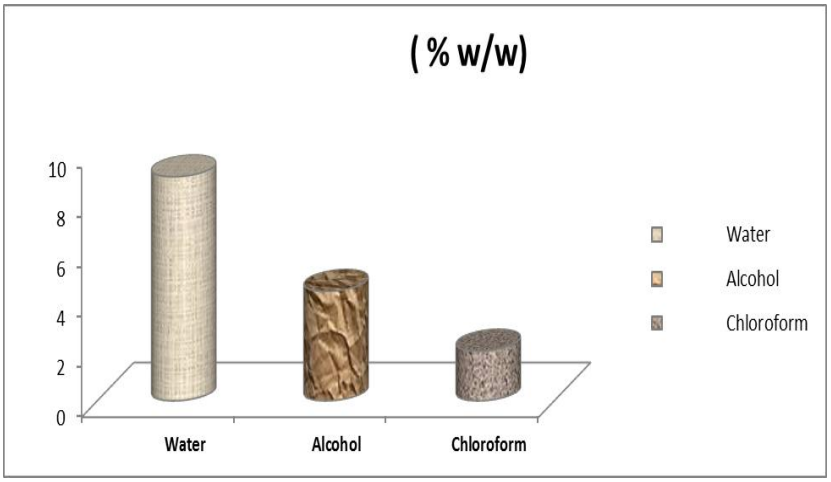


Figure. 9: Comparative study among the extractive values.

DISCUSSION

In this view, pharmacognostical evaluation of *Cyperus kyllingia* is necessary. According to the WHO,determining the macroscopic and microscopic characteristics are the first steps towards establishing the identity and purity of materials used in the plant and these steps should be carried out before any further tests are undertaken. The quantitative determination of physicochemical parameters are useful for setting standards for crude drugs. Ash content analyses indicate the degree of admixture of foreign inorganic matter either from the storage container or by intentional addition to disguise the appearance of the crude drug. The extactive values are primarily useful for the determination of the exhausted or adultrated drug.[30,31] In this pharmacognostical study we observed different organoleptic parameter and also observed different microscopic characters like, the pith is larger, the vascular bundles varies from 6-8, intercellular space is absent in between endodermis, this shows that it is a monocot root. In the present project we determine the different ash values & extractive values of the *C. kyllinga* root. In recent years, there has been a rapid increase in the standardization of selected medicinal plants with significant potential as therapeutics due to their specific healing properties and potential actions.

CONCLUSION

After the present investigation it can be concluded that the pharmacognostical studies of the *Cyperus Killingia* yielded a set of qualitative and quqntitative parameters or standards that can serve as an important source of information to ascertain the identify and to determine the quality and purity of the plant materials for future studies. These parameters also will serve as standard data for quality control studies of pharmaceutical preparations from the *Cyperus killingia* root.

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