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PHYTOCHEMICAL ANALYSIS AND IDENTIFICATION OF CONSTITUENTS PRESENT IN MEDICINAL PLANT *SPHAERANTHUS INDICUS*

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ABSTRACT:- *Sphaeranthus indicus Linn*, commonly known as "Gorakhmundi" or "East Indian Globe Thistle," is a medicinal plant that has been traditionally used in various systems of medicine for its therapeutic properties. It belongs to the Asteraceae family and is widely distributed in India, Southeast Asia, and other tropical regions. The plant has gained attention due to its potential health benefits and is known for its diverse phytochemical composition. Phytochemical analysis plays a crucial role in identifying and quantifying the bioactive compounds present in plants. Various classes of phytochemicals, such as alkaloids, flavonoids, terpenoids, phenolic compounds, and others, are known to exhibit antioxidant properties and contribute to the plant's medicinal properties. This study aims to conduct an in vitro phytochemical analysis and evaluate the antioxidant activity of *Sphaeranthus indicus Linn*. The present study aim's to determine the phytochemical analysis and Antioxidant activity of *Sphaeranthus indicus linn.* This study's effective isolation and extensive characterization of apigenin provide the groundwork for future research into its pharmacological actions. The structural insights gained through advanced spectroscopic techniques imply that apigenin has potential for usage in a variety of therapeutic applications, particularly in the fields of oxidative stress, inflammation, and cancer treatment.

KEYWORD:- *Sphaeranthus indicus,* Phytochemical analysis and Medicinal value.

INTRODUCTION:-

Herbal medicines have been used by humans from time immemorial. Ayurveda, India's oldest traditional system, demonstrates that ancient Indians had extensive understanding of the medicinal properties of several plants (Kirtikar and Basu, 1981). India has an extremely rich flora due to the great variances in climatic and geographical circumstances that exist throughout the country. Many of the crude medications utilised in traditional systems have been scientifically studied (Chatterjee and Pakrashi, 2003).

For thousands of years, plants have played an important part in sustaining human health and improving the quality of human existence. They are also valuable components of medications, flavours, beverages, cosmetics, and dyes. Herbal therapy is founded on the idea that plants contain natural chemicals that can improve health and treat illness (Dhara et al., 1968). In recent years, there has been an increased emphasis on plant study all over the world, and a vast body of evidence has been gathered to demonstrate the enormous potential of medicinal plants employed in diverse traditional systems. Today, there is a surge of public interest in the usage of herbal treatments.

Furthermore, many western drugs originated from plant extracts. There are numerous herbs that are commonly used to treat cardiovascular difficulties, liver illnesses, central nervous system, digestive and metabolic disorders. Given their ability to have considerable therapeutic effects, they could be used as a medicine or supplement in the treatment and management of a variety of disorders. Such have been and continue to be used as folk medicine or food supplements for a variety of diseases (Chopra et al., 1956). Ethnopharmacological studies on such herbs and medicinal plants continue to pique the curiosity of researchers all around the world. Sphaeranthus indicus is one such plant that has piqued the interest of experts around the world due to its biological properties. Sphaeranthus indicus Linn belongs to the family Asteraceae and is a medicinally important plant used in

folk medicine (Ambavade*et al.*, 2006).

Isolation of bioactive phytochemicals from plants is an important step in understanding their therapeutic capabilities. Column chromatography is one of the most extensively utilised procedures for separating and purifying complicated phytochemical mixtures. This approach employs a stationary phase, commonly silica gel, and a mobile phase composed of solvents of varied polarity to accomplish efficient separation of distinct compounds depending on their interactions with the phases(Sasidharan*et al.*, 2011).

In the case of *Sphaeranthus indicus*, column chromatography was used to isolate important phytoconstituents. These substances are identified using a mix of techniques, including thin-layer chromatography (TLC), nuclear magnetic resonance (NMR), and mass spectrometry (MS) (Dhanani et al., 2017). Fractionation allows for the isolation of pure molecules, which are then tested for medicinal potential using bioactivity assays. This methodical technique ensures that the active components responsible for *S. indicus'* pharmacological actions are accurately identified.

OBJECTIVE OF THE STUDY:-

The discipline whose main objective is the study of the chemical constituents of plants is Phyto-chemistry. The study of such compounds includes: their chemical structures, metabolism (biosynthesis and degradation), natural distribution, biological function, extraction and qualitative-quantitative evaluation. Before starting, any phytochemical analysis is important to have an adequate preparation of our plant material. Phytochemical research of a plant includes several aspects:

- Extraction of the compounds to be analyzed from a sample.
- Separation and isolation of them.
- Identification and/or characterization of the isolated compounds.
- To phytochemical investigation of root and stem part by various chemical tests.
- Chemical investigation for the medicinal plant is to contributed and correlated the activity of this plant and the chemical composition phenolic compounds flavonoidsm flovonols
- Investigation of the biosynthetic routes of a certain molecule.
- Determination or quantitative assessment.
- Various traditional books described that whole plant is medicinally used so there is required to find out either underground part or aerial part is more active for Antioxidant activity, Analgesic activity, Diuretic activity and Anti-inflammatory activity.

REVIEW OF LITERATURE:-

Sphaeranthus indicus Linn. belongs to family Asteraceae. The plant is commonly known as Gorakhmundi. It is an annual spreading herb, which grows approximately 15-30 cm in height. The plant is distributed throughout the plains and wet lands in India, Sri Lanka and Australia (Gogate, 2000). All the parts of the plant have medicinal uses. Plants have a great potential for producing new drugs of great benefit to mankind. Essential oil obtain by steam distillation of the whole herb contains ocimen (Baslas, 1959), obtained by steam distillation of the whole herb contains ocimen, α- terpine, a- citral, geranion, aionone, β-ionone, d- cadinene, p- methoxy cinnamaldehyde (Basu et al., 1946) and an alkaloid spearanthine (Gupta et al., 1967). The alcoholic extract of powdered caputula contains stigmasterol, β- sitosterol, hentriacontane, sesqueterpinelactone, sphaeranthanolide flavone and isoflavone glycoside (Yadav et al., 1998). In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias (Kirtikar and Basu, 1978). There are several reports on the antimicrobial activity of different extracts and biologically active compounds isolated from plant species used in herbal medicine (Yuldasheva et al., 2005).

According to Ayurveda, this herb is used in medaroga, laxative, digestible, tonic, alterative, anthelminitic and alexipharmic (Gupta, 1984). It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. *Sphaeranthus*

indicus Linn showed good anti diabetic activity (Kharkar et al., 2013).

Considering the aforesaid, one of the traditionally used medicinal plants belonging was screened for their antimicrobial properties. *Sphaeranthus indicus* Linn. belongs to the family Asteraceae. It is used in homeopathic medicine for the treatment of insomnia, epilepsy, tetanus, muscle spasms and leaves presented anxiolytic activity (Ambavade et. al 2006; Amarasingam et. al. 1964). *Sphaeranthes indicus Linn*. is an annual spreading herb used to treat hemicraniasis (Chopra et. al. 1958), jaundice, diabetes, hernia, haemorrhoids, helmenthiasis, skin diseases, nervine tonic etc. the bark ground and mixed with whey is useful in treating piles. Leaf juice is boiled with milk and sugar prescribed for cough. An aqueous extract of the whole plant was slightly toxic to American cockroaches (Das and Bhattacharjee, 1970). Sphaeranthus indicus was found to possess powerful medicinal properties to cure diseases of liver, bronchitis, jaundice and skin diseases. In view of the medicinal importance of *Sphaeranthus indicus* Linn. in the indigenous system, it was decided to work on the phyto-chemistry and antimicrobial investigation on *Sphaeranthus indicus* Linn.

MATERIAL AND METHODS:-

Collection of *Sphaeranthus indicus*

Sphaeranthus indicus (Root and Stem) was taken from the local area of Bhopal in July, 2021. Fresh plant materials were dried in the shade. Dried plant pieces were stored in plastic bags, sealed tightly, and then powdered according to the specifications.

Extraction by maceration method

Maceration is a simple and commonly used procedure for extracting phytochemicals from plant materials, especially those that are heat-sensitive or rapidly destroyed. This process involves soaking the plant material in a suitable solvent at room temperature for a lengthy period of time, allowing the solvent to permeate the plant tissues and dissolve the bioactive chemicals (Handa et al., 2008).

Determination of percentage yield

Percentage yield assesses the general effectiveness of the extraction process. It compares how much product a researcher obtained after running the methods to how much was really obtained. A higher percentage yield indicates that the researcher acquired more product following extraction. The formula for calculating percentage yield is as follows:

$$\textbf{PercentageYield} = \frac{Weight of Extract}{Weight of Powder drug taken} x\ 100$$

Isolation of compound from ethyl acetate extracts of stem part of *Sphaeranthus indicus*

Optimization of Thin layer chromatography of ethyl acetate extract of stem part of *Sphaeranthus indicus* extract

Thin layer chromatography is based on the adsorption process. In this type of chromatography, the mobile phase, which contains the dissolved solutes, travels over the stationary phase's surface.

Detection and Calculation of R_f Value

Once the chromatogram was created, the Rf value of the spot was determined using the formula, and the results are shown in Table 1.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

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TLC Optimization ethyl acetate extract of *Sphaeranthus indicus* extract

Table 1: TLC Optimization ethyl acetate extract of *Sphaeranthus indicus* stem extract

S. No.	Mobile phase	Spot Distance	Rf Value
1	**Toluene: Ethyl acetate (7:3)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 4 No. of spot at short UV- 5 No. of spot at normal light- 1	 1.5, 2.9, 3.9, 5.1 1.5, 2.9, 3.6, 4.4, 5 1.5	 0.25, 0.48, 0.65, 0.85 0.25,0.48,0.6,0.73, 0.83 0.25
2	**Toluene: Ethyl acetate (6:4)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 1 No. of spot at normal light- 1	 1 1 0.9	 0.16 0.16 0.15
3	**Toluene: Ethyl acetate (8:2)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 1 No. of spot at normal light- 1	 2.5 2 1.4	 0.41 0.33 0.23
4	**Toluene: Ethyl acetate (5:5)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 1 No. of spot at normal light- 1	 1 1.5 1	 0.16 0.25 0.16
5	**Chloroform: Methanol (9:1)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 2 No. of spot at normal light- 1	 5.5 5, 5.5 2	 0.91 0.83, 0.91 0.33
6	**Chloroform: methanol (6:4)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 1 No. of spot at normal light- 1	 5.5 5.5 5.5	 0.91 0.91 0.91
7	**Chloroform: Methanol (5:5)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 1 No. of spot at normal light- 1	 5.5 5 5.5	 0.91 0.83 0.91
8	**Chloroform: Methanol (7:3)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 1 No. of spot at short UV- 1 No. of spot at normal light- 1	 5.5 5.5 5	 0.91 0.91 0.83

RESULTS AND DISCUSSION:-

Results of % Yield of *Sphaeranthus indicus*

Table 2: % Yield of *Sphaeranthus indicus*

Sr. No.	Extracts	% Yield (W/W)
	Root extract	
1	Chloroform	0.9%
2	Ethyl acetate	4.8%
	Stem extract	
1	Chloroform	1.6%
2	Ethyl acetate	5.2%

The yield from chloroform in root extracts is minimal, at 0.9%. This implies that chloroform is ineffective for extracting bioactive chemicals from the root of *Sphaeranthus indicus*. Chloroform is a non-polar solvent that is more effective at extracting non-polar substances. The low yield suggests that the root has a high concentration of highly polar chemicals that chloroform cannot efficiently extract. On the other hand, the yield of ethyl acetate extraction is significantly higher, at 4.8%. Ethyl acetate is a moderately polar solvent that may enable for the extraction of a wider spectrum of molecules, including both polar and non-polar components. This demonstrates that ethyl acetate is more suited to extracting bioactive compounds from the root, and the higher yield indicates that more of the plant's active components are soluble in this solvent. For stem extracts, the chloroform extract yields 1.6%, which is more than the root extract but still lower than the ethyl acetate extraction. This could indicate that chloroform is more successful in extracting substances from the stem than from the root, potentially due to the stem's higher amount of non-polar molecules. The yield remains lower as compared to ethyl acetate, indicating that chloroform is still less efficient for overall extraction.

Results of Phytochemical Test of *Sphaeranthus indicus*

Table 3: Phytochemical Test of *Sphaeranthus indicus* (Root) extract

Sr. No.	Test	Chloroform	Ethyl acetate
1.	Carbohydrate Fehlings Test Benedicts Test	 - ve - ve	 + ve + ve
2.	Flavonoids Lead acetate Test Alkaline Test	 - ve - ve	 - ve - ve
3.	Phenols Ferric chloride Test	 - ve	 - ve
4.	Saponins Foam Test	 - ve	 - ve
5.	Proteins Xanthoproteic Test	 - ve	 - ve
6.	Diterpenes Copper Acetate Test	 - ve	 - ve
7.	Alkaloid Wagner’s Test	 - ve	 + ve
8.	Glycosides Conc. Sulphuric acid Test	 - ve	 - ve
9.	Lignin		

	Labet Test	- ve	- ve
10.	Sterols Salkowski Test	- ve	- ve
11.	Tannins Gelatin Test	- ve	- ve

[+ ve = positive; - ve = negative]

Table 4: Phytochemical Test of *Sphaeranthus indicus* (Stem) extract

Sr. No.	Test	Chloroform	Ethyl acetate
1.	Carbohydrate Fehlings Test Benedicts Test	 + ve + ve	 + ve + ve
2.	Flavonoids Lead acetate Test Alkaline Test	 + ve + ve	 + ve + ve
3.	Phenols Ferric chloride Test	 +ve	 + ve
4.	Saponins Foam Test	 - ve	 - ve
5.	Proteins Xanthoproteic Test	 - ve	 + ve
6.	Diterpenes Copper Acetate Test	 + ve	 + ve
7.	Alkaloid Wagner's Test	 - ve	 +ve
8.	Glycosides Conc. Sulphuric acid Test	 - ve	 - ve
9.	Lignin Labet Test	 - ve	 - ve
10.	Sterols Salkowski Test	 - ve	 - ve
11.	Tannins Gelatin Test	 - ve	 - ve

[+ ve = positive; - ve = negative]

The phytochemical screening of *Sphaeranthus indicus* stem extracts using chloroform and ethyl acetate solvents revealed the presence of different bioactive chemicals, which are significant for understanding the plant's potential therapeutic capabilities.

In terms of carbohydrates, both the chloroform and ethyl acetate extracts accepted Fehling's and Benedict's tests, showing the existence of reducing sugars or carbohydrates. This shows that carbohydrates, which are essential for energy storage and other cellular functions, exist in the stem.

Results of total flavonoids content

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$y = 0.032x + 0.002$, $R^2=0.999$**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 5.: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.167±0.003
2	10	0.335±0.002
3	15	0.495±0.003
4	20	0.654±0.004
5	25	0.823±0.002

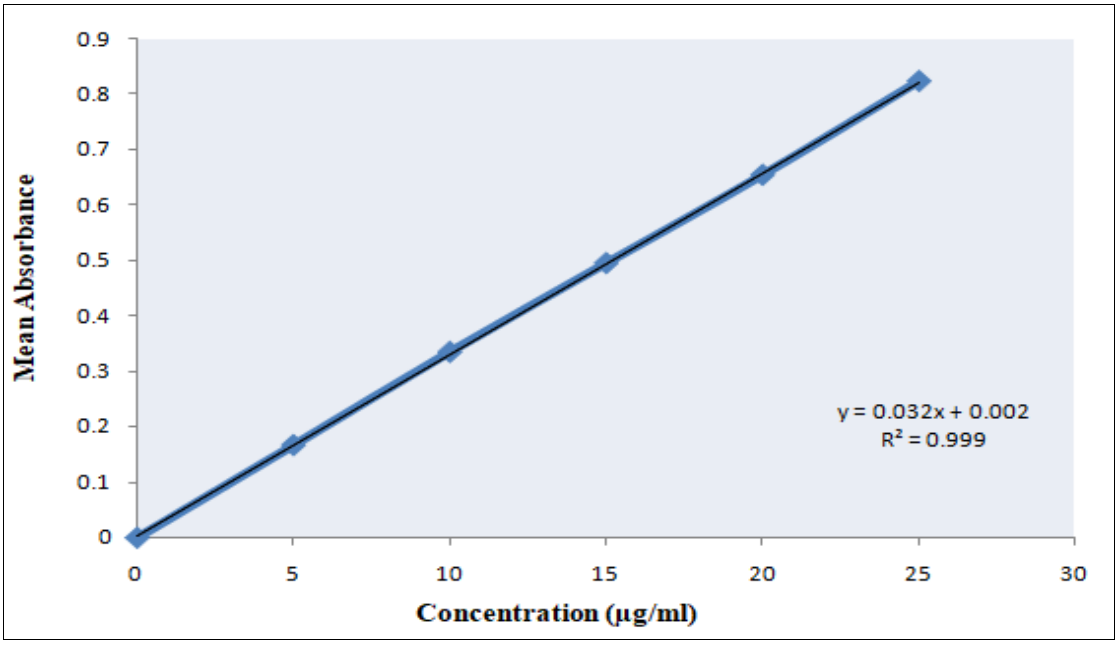

Figure 1.: Graph of calibration curve of Quercetin

Results of total phenolic content (TPC)

Total phenolic content (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **y = 0.019x + 0.021, R^2= 0.998,** where X is the Gallic acid equivalent (GAE) and Y is the absorbance.

Table 6: Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.227±0.003
2	20	0.435±0.004
3	30	0.626±0.003
4	40	0.812±0.002
5	50	0.993±0.003

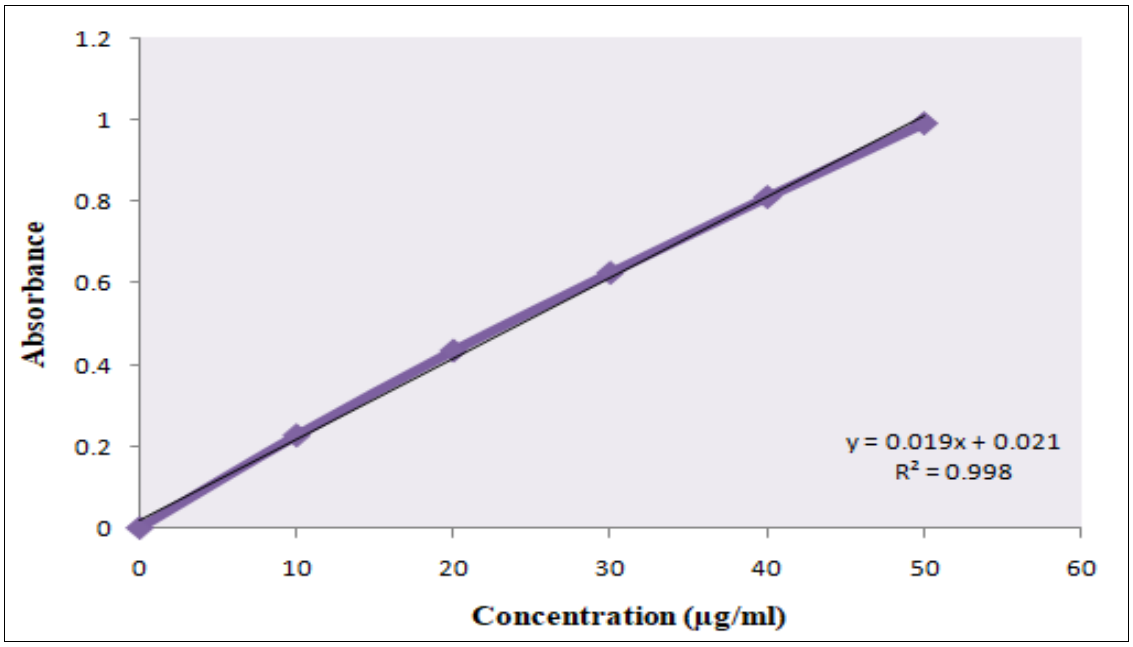

Figure 2: Graph of calibration curve of Gallic acid

Table 7: Absorbance total flavonoids and phenol content in *Sphaeranthus indicus*

S. No.	Extracts	Root part	Stem part
		Total flavonoids content	
1.	Chloroform	-	0.145
2.	Ethyl acetate	-	0.265
		Total phenol content	
1.	Chloroform	-	-
2.	Ethyl acetate	-	0.125

Table 8: Results of total flavonoids and phenol content in *Sphaeranthus indicus*

S. No.	Extracts	Root part	Stem part
		Total flavonoids content	
1.	Chloroform	-	0.446mg/100mg
2.	Ethyl acetate	-	2.550mg/100mg
		Total phenol content	
1.	Chloroform	-	-
2.	Ethyl acetate	-	0.504mg/100mg

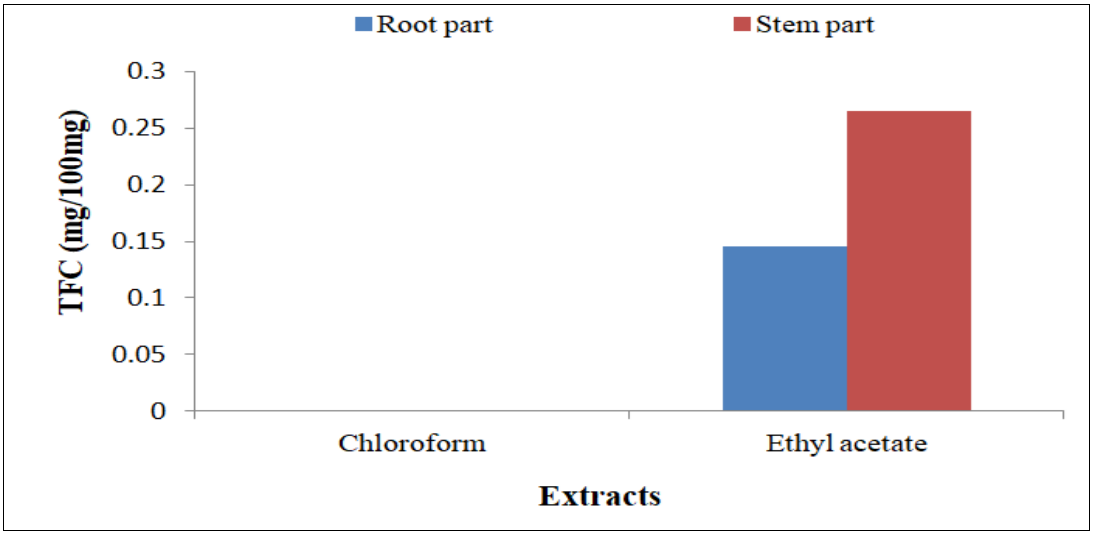

Figure 3: Graph of total flavonoids content in *Sphaeranthus indicus*

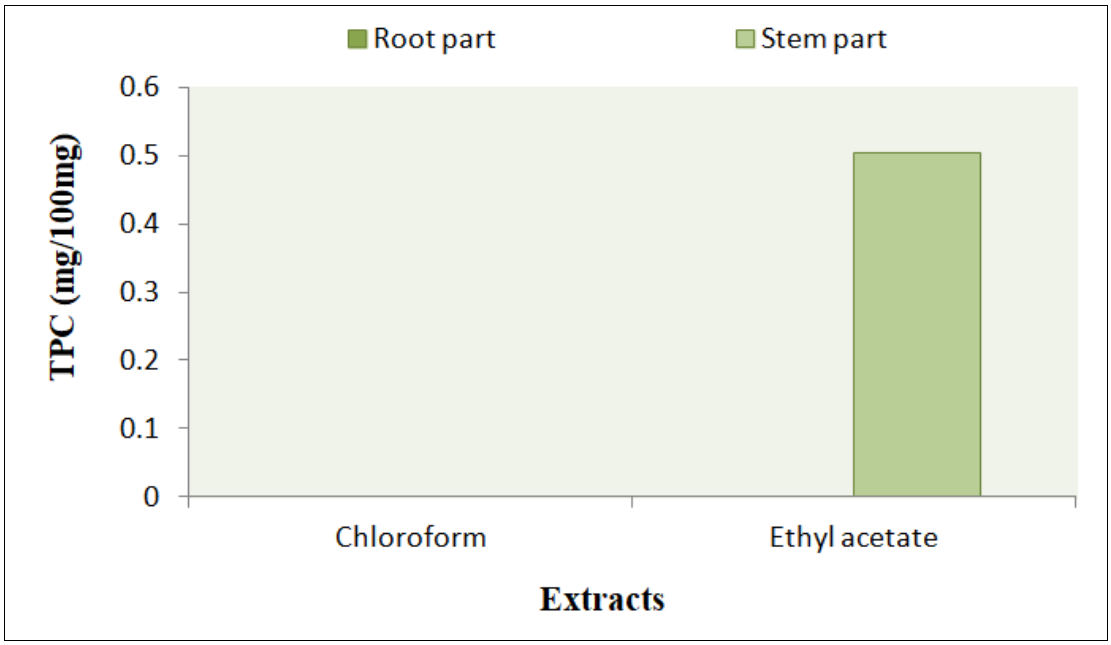

Figure 4: Graph of total Phenol content in *Sphaeranthus indicus*

Flavonoids were absent from the root part of the chloroform extract, but were found in the stem component at a concentration of 0.446 mg/100 mg. However, no phenolic compounds were detected in either the root or stem of this extract. In contrast, the ethyl acetate extract showed a higher flavonoid content, particularly 2.550 mg/100 mg in the stem. Again, while phenols were absent in the root, they were present in the stem at a concentration of 0.504 mg/100 mg. These findings indicate that *Sphaeranthus indicus*, particularly its stem, is a rich source of flavonoids. Flavonoids are well-known for their antioxidant characteristics and potential health benefits, which suggests that this plant extract has therapeutic

potential. However, the absence of phenols in the root and their low concentration in the stem requires more research into the specific chemicals present and their biological properties.

Results of isolation and identification

Table 9: Selection of mobile phase

S. No.	Mobile Phase	Observation
1.	Toluene: Ethyl acetate (7:3)	Most Suitable
2.	Toluene: Ethyl acetate (6:4)	Not proper resolution
3.	Toluene: Ethyl acetate (8:2)	Not proper resolution
4.	Toluene: Ethyl acetate (5:5)	Not proper resolution
5.	Chloroform: Methanol (9:1)	Not proper resolution
6.	Chloroform: methanol (6:4)	Not proper resolution
7.	Chloroform: Methanol (5:5)	Not proper resolution
8.	Chloroform: Methanol (7:3)	Not proper resolution

The selection of the mobile phase is an important stage in chromatographic procedures since it directly impacts the separation and resolution of the chemicals being analysed. Among the investigated mobile phase combinations, Toluene: Ethyl acetate (7:3v/v) showed to be the most suited, providing the best resolution and component separation. This ratio appears to be the best proportion for optimal chromatographic performance. Other toluene:ethyl acetate ratios, such as 6:4, 8:2, and 5:5, could not provide adequate resolution, resulting in poor separation. Similarly, Chloroform:Methanol combinations at various ratios (9:1, 6:4, 5:5, 7:3v/v) could not produce adequate resolution, indicating that these solvent mixes were unsuitable for effective separation. The 7:3 ratio of toluene and ethyl acetate proved to be the most effective mobile phase, with the other combinations failing to achieve good resolution in chromatographic separations.

CONCLUSION:-

The present study regarding the traditional uses of phytochemicals, activities of crude extracts as well as pure compounds, analysis of active compounds, related to , *Sphaeranthus indicus*. Although the chemical structure and its biological potential of some of the constituents are known, generally, the mechanisms of action need to be investigated for further development into therapeutics. Natural products have been and are still a major plank in supporting the primary health systems. Their bio-activity is mainly associated with secondary metabolites, often elaborated for the plant defense. Some of these phytochemicals accidentally protect humans against pathogens and that is why they are a main target for drug prospecting programs. These phytochemicals are known to have several properties important to cells including; prophylactic, therapeutic, nutritive and immune-modulative properties. This study's effective isolation and extensive characterization of apigenin provide the groundwork for future research into its pharmacological actions. The structural insights gained through advanced spectroscopic techniques imply that apigenin has potential for usage in a variety of therapeutic applications, particularly in the fields of oxidative stress, inflammation, and cancer treatment.

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