Biological Forum – An International Journal **15(3): 444-449(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Phytochemical Screening and Biological activities of *Eleusine indica* leaf extract

B. Jone Magadelin[1*], S. Ajith Sinthuja[2] and S. Jameer Ahamed[3]
[1]Ph.D. Research Scholar (Autonomous), Nagercoil-04,
Affiliated to Manonmaniam Sundaranar University,
Abishekapatti, Tirunelveli (Tamilnadu), India.
[2]Assistant Professor, Department of Chemistry,
Holy Cross College (Autonomous), Nagercoil (Tamilnadu), India.
[3]Ph.D. Research Scholar, Centre for Marine Science and Technology,
Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli (Tamilnadu), India.

(Corresponding author: B. Jone Magadelin)*
(Received: 12 January 2023; Revised: 15 February 2023; Accepted: 22 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: The presence of secondary metabolites in medicinal plants is useful for healing as well as for curing human diseases. Most of the synthetic drugs being marketed have more vulnerable side effects, which in turn creates some biological and environmental collisions when discarding the expired pharmaceutical synthetic products. Nowadays, traditional approaches are emerging for the treatment of several diseases and disorders using medicinal plant extracts. The current study was anticipated to appraise the secondary metabolites and their capabilities by means of crude ethanolic leaf extracts of *Eleusine indica*. For phytochemical screening, customary procedures were used, considering Mayer's test, the shinoda test, the salkowski test, the Keller-Killiani test, and the foam test, which were worn to determine total alkaloids, flavonoids, steroids, glycosides, and saponins. Radical scavenging DPPH and the nitric oxide scavenging assay were used to appraise anti-oxidant activity. Antimicrobial activity was determined using a standard disc diffusion assay, while cytotoxicity was determined using MCF-7 cell lines. FT-IR spectroscopy was employed to collect spectral details. Alkaloids, flavonoids, steroids, glycosides, and saponins were inspected positively in the preliminary phytochemical screening. TFC, TAC, and TSC's in leaf extracts obtained were 26.4 mg/ml, 20.58 mg/ml, 36.0 mg/ml, and 283.8 mg/ml, respectively. The DPPH free radical scavenging activity of the ethanolic extract of *Eleusine indica* showed 82.25% (100 µg/ml). The crude extract's maximum in-vitro nitric oxide scavenging activity was 40.54% at 100 g concentration. In *Pseudomonas aeruginosa*, the maximum zone of inhibition was found to be 16 mm, and maximum cytotoxicity with an IC50 value of 85.09% by *Eleusine indica* were the best calculated values. This work rationalizes the use of secondary metabolites and their plausible action against microbes for the unfolding of new drugs. In the future, distinctive *in vivo* and *in vitro* consanguineous studies can be accomplished in addition to examining its western medical applications.

Keywords: Phytochemical profiling, *Eleusine indica*, Anti-oxidant, Anti-bacterial, Cytotoxicity, FT-IR, Molecular docking.

INTRODUCTION

Eleusine indica generally considered a hemp species, is indigenous to the tropics and subtropics (Haber and Semaan 2007). *Eleusine indica* also called gooseberry, is a depurative, diuretic, febrifuge, and laxative, and therefore is used for the treatment of influenza, hypertension, oliguria, and urine retention. The plant has been a component of the "basic remedy" in traditional Vietnamese medicine and is also used for kidney problems in Trinidad and Tobago (Al-Zubairi *et al*., 2011). The seed of *Eleusine indica* is sometimes used as a famine food and in the treatment of liver complaints (Iqbal and Gnanaraj 2012). The locals of Kadazan-Dusun in Sabah have also used an aqueous extract made from infusing E. indica aerial parts with rice to treat flu-related symptoms (Piah, 2020). Two additional phytochemicals, p-coumaric acid and isoschaftoside, as well as a number of primary metabolites and amino acids, have been identified in a recent study on the plant extract using metabolite fingerprinting and profiling (Pealoza *et al*., 2018). According to the results of the phytochemical analysis of the *E. indica* extract, alkaloids, terpenes, flavonoids, tannins, anthraquinones, saponins, and cardiac glycosides are present (Okokon *et al*., 2010). Pharmacologically, *Eleusine indica* is believed to have anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, anti-plasmodial, anti-diabetic, and anti-cancer potential activities (De Melo *et al*., 2005; Nas *et al*., 2020).

To outline the UV-damaged DNA-binding protein complex, Damaged DNA-binding protein 1 (DDB1) exhibits the nucleotide excision pathway and is needed for DNA repair. DDB1 was anticipated to be involved in apoptosis and chemo-resistance regulation in different cancers. It is needed for DNA repair, and it frequently binds to DDB2 to form the UV-damaged

DNA-binding protein and to perplex proteins of the nucleotide excision pathway to start DNA repair (Yeh *et al*., 2012). The cancellation of DDB1 abrogates the self-renewing capacity of hepatocytes and implements the compensatory proliferation of DDB1-expressing hepatocytes, thus leading to hepatocellular carcinoma (Yamaji *et al*., 2010). Liu *et al*. (2017) found that thyroid transcription factor 1 could interact with DDB1 and block its binding to checkpoint kinase 1 (CHK1), which attenuated the ubiquitylation, manually inducing CHK1 degradation, and contributing to lung adenocarcinoma development (Liu *et al*., 2017). At last, a recent study implemented that DDB1 and Cullin-RING ubiquitin ligases (CRL) 4, the ubiquitin ligase of Cullin 4A (CUL4A)-DDB1 E3, are important factors in ovarian cancer chemo-resistance because they normalise apoptosis and are therefore therapeutic targets for patients after cis-platin failure (Hu *et al*., 2019). In the current study, research has been conducted on *Eleusine indica* as low effective cost, natural source. The anti-microbial and anti-oxidant activity of ethanolic leaf extracts were performed.

MATERIALS AND METHODS

Plant collection and extract preparation. Green and mature leaves of *Eleusine indica* were gathered from Veliavilai, Kanniyakumari District (Tamil Nadu, India), and cast off for the composition of the extract. The secondary metabolites were extracted using the ethanol solvent extraction method through a Soxhlet apparatus. 50 grammes of dried and powdered selected plant leaf samples were packed in an extraction bung and placed into the soxhlet tube, followed by the extraction with 200 mL of ethanol. Afterwards, the extract was heated in a hot water bath at 55-60°C until all the solvents got evaporated so as to extract the bioactive compounds. This was kept undisturbed for 24 hours. After 24 hours, the extract was collected and filtered to discard the particles with larger sizes, and the yield was found to be 0.68gm. Then the extract was stored at 4ºC for further studies.

Phytochemical profiling. Phytochemical screening of the ethanol fraction of *Eleusine indica* leaves was executed to confirm the existence or absence of alkaloids by Mayer's test, flavonoids by Shinoda test, steroids by Salkowski test, glycosides by Keller-Killiani test, and saponins by foam test using the strategies described by Harborne (1998); Trease and Evans (1996); Robert *et al*. (1971).

Total alkaloids, flavonoids, steroids, glycosides and saponins contents

Mayer's test: 1ml of the extract treated with 3ml of Mayer's reagent. The yellow precipitate emergence indicates alkaloids existence (Ansari, 2006).

Shinoda test: To 5ml of the extract, an equal volume of 95% ethanol and dilute hydrochloric acid along with a small piece of magnesium chloride were adjoin and the solution was boiled for five minutes. The reddish-pink existence of color indicates the presence of flavonoids (Kokate 1994).

To 2ml of the extract, 2ml of chloroform and concentrated sulphuric acid were poured. The lower chloroform layer produced red color that indicated the existence of steroids. Other test was exhibited by mixing 2ml of acetic acid with concentrated sulphuric acid and extracted with 2ml of chloroform. The greenish-yellow fluorescence indicated the entity of steroids (Indian Pharmacopoeia 1996).

Borntrager's test: To 2ml of the extract, 2ml of chloroform was added and shaken vigorously. Once the chloroform layer gets individual a same volume of dilute ammonia was added. The formation of yellow color indicates the presence of glycosides (Evans, 1996).

Foam test: To 0.05ml of the extract, 5ml of distilled water was added and shaked vigorously for a stable persistent froth. Frothing which persisted on warming exhibits the existence of saponins (Ansari 2006).

Fourier transform infrared spectroscopy analysis (FT-IR). In the current study, extract was employed to analyse the functional characteristics, and Kemp's Fourier transform infrared (FTIR) approach was used to evaluate the existence of bonding of functional groups in the ethanolic extract of *Eleusine indica.* The dried samples of *Eleusine indica* extract (0.1 - 2.0 by weight) were ground with KBr and compressed into a clear water or disc to create KBr discs. The KBr was dried, so it was advantageous to grind under an infrared lamp to prevent air moisture condensation, which results in wide absorption at 3500/cm. The KBr sample complex was ground to a particle size of 2 m to prevent wavelength scaling. High pressure caused the KBr disc to fairly condense to a 13 mm diameter and 0.3 mm thickness. The vibration spectrum was captured as a graphical chart, and its frequency ranged from 400 to 5000/cm wave number. Japan's Shimadzu manufactured the FTIR analysis device.

Anti-microbial activity (disc diffusion method). The medium was developed by dissolving 38g of Muller-Hinton Agar Medium (Hi-Media) in 1000 ml of distilled water. It was autoclaved for 15 lb of pressure at 12100C for 15 minutes (pH 7.3). The medium was cooled, mixed well, and poured. Petriplates (25ml/plate) were swabbed with pathogenic bacteria cultures, viz., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris.* Finally, about 25µL, 50µL and 75µL were loaded onto the disc and then placed on the surface of the Mullar-Hinton medium. The plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were analysed around the disc and examined with a transparent ruler in millimetres. The size of the zone of inhibition (including the disc) was measured in millimetres. The absence of zone inhibition was guessed as the absence of activity (Kohner *et al.,* 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition is less than 7 mm, intermediate (8-10 mm), and sensitive if it is more than 11 mm (Assam *et al*., 2010).

***In vitro* antioxidant activities**

Free radical scavenging activity by DPPH. The scavenging of DPPH radicals was set in motion by the approaches of Brand-Williams *et al*. (1995); Sanchez-Mareno (2002) with minor modifications. The DPPH solution was obtained by getting 7.89 mg of DPPH using a chemical balance and it was dissolved with 100

ml 99.5% ethanol. Further it was placed in dark for about 2 hours. In the mean time, a DPPH solution of 1,000µl was added to 800µl of Tris-HCl buffer (pH 7.4) in a testing tube. Afterwards, 200µl of testing sample solution was added on and it was mixed quickly. The solution was kept at room temperature for 30 min. The absorbance of the solution at 517 nm was recorded. A mixed solution with 1,200µl of ethanol and 800 µl of Tris- HCl buffer (pH 7.4) was used as the blank. The ability of the crude extract and synthesized gold nanoparticles to scavenge DPPH radicals was determined by the following equation:

% of DPPH Radical Scavenging Activity (% RSA) =

$$\frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Absorbance control is the observance of DPPH radical + ethanol

Absorbance sample is the observance of DPPH radical + extract

IC_{50} values were calculated as the average of triplicate analyses.

Radical scavenging activity by nitric oxide

The nitric oxide scavenging activity was unfaltering as stated to the modified method of Alderson *et al.* (2001). 1ml of Sodium nitroprusside (10mM) in phosphate-buffered saline was assorted with the 1ml of varied concentrations of leaves (20, 40, 60, 80 and 100 µg/ml) and incubated at 25°C for 180 minutes. To the incubation solution, 1 ml of Griess reagent (prepared by mixing equal volumes of 1% sulphanilamide, 0.1% naphthyl ethylenediamine dichloride, and 3% phosphoric acid) was added and the absorbance was read at 546nm. The standard Ascorbic acid was cast-off as a pragmatic control tended in the same way with Griess reagent. The inhibition (%) was deliberated using the formula:

$$\text{Scavenging Activity } \% = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Anti-cancer activity. The activity of plant leaf extract against the MCF-7 human breast adenocarcinoma cell line was performed through MTT assay. For variety of attempts, Human MCF-7 cells deed as a model for breast cancer cell growth. The metastatic breast cancer in 1970 was the first identified cell tested for 69 years old patient. MCF-7 cell line was the first new cell line, which response promptly to estrogens. The utterance of receptors for estrogen and progesterone was varied in MCF-7 cells and its sensitivity to anti-estrogens and anti-progesterone is flaunted in various ways (Hamelers *et al.,* 2003). For the determination of cytotoxicity, MTT assay was used. The cytotoxicity screening model dispense important particulars mainly on the activity of crude extracts and fractions on the premise of which forthcoming work can be assigned (Ferguson *et al.,* 2004).

RESULT AND DISCUSSION

Phytochemical Screening. From the plant leaves of *Eleusine indica,* the phytochemical constituents such as alkaloids, flavonoids, steroids, glycosides and saponins were present while carbohydrate, protein, phenols and tannins were absent.

Total alkaloids, flavonoids, steroids, glycosides and saponins contents. The plants pharmacological potential is determined by secondary metabolites. The total flavonoid content obtained for *Eleusine indica* was 26.4mg/ml, the total alkaloid content obtained was 20.58mg/ml, the total steroid content obtained was 36.0mg/ml and the total saponin content obtained was 283.8mg/ml. Chemical substances such as phenols, anthraquinones, coumarins, essential oils, triterpenes, steroids, fatty acids, tannins, flavonoids, alkaloids, and coumarins have been discovered and isolated (Ette O Ettebong *et al.*, 2020).

FT-IR Spectroscopy. The identification of the functional groups of the bioactive components present in plant leaf extract was carried out by FT-IR Spectroscopy which is based on the peak values in the region of IR radiation. In the current study, functional groups were analysed with the help of FT-IR spectroscopy based on the absorbance (Table 1). The peaks obtained from the analysis were determined. The functional groups in the leaf extract refer to a specific class of compounds (Fig. 2).

Table 1: FT-IR Spectral data of gold nanoparticle synthesized from *Eleusine indica.*

Sr. No.	Peak	Intensity	Characteristic Absorptions (cm^{-1})	Possible Functional Group	Specific Context
1.	518.82	94.669	680-500	C-Br stretch	Alkyl halide
2.	924.8	99.306	950-910	O-H bend	Carboxylic acid
3.	1510.16	95.096	1525-1515	N-O nitro comp. Stretching	Nitro group
4.	1641.31	93.699	1645-1637	C=C stretch	Alkenes
5.	2064.66	93.214	2140-1990	N=C in R-N=C=S	R-N=C=S
6.	3446.56	29.056	3455-3445	NH stretch	Aryl Amines

Table 2: % cell viability values and observed IC_{50} value of the given sample, (*Eleusine indica*) against MCF-7 cells after the treatment period of 24hrs.

Culture condition	% cell viability	IC_{50}conc (µg/ml)
Untreated	100	**85.09**
Std control (Dox-1uM)	26.00	
10µg/ml	91.91	
25µg/ml	86.42	
50µg/ml	79.49	
75µg/ml	60.98	
100µg/ml	34.09	

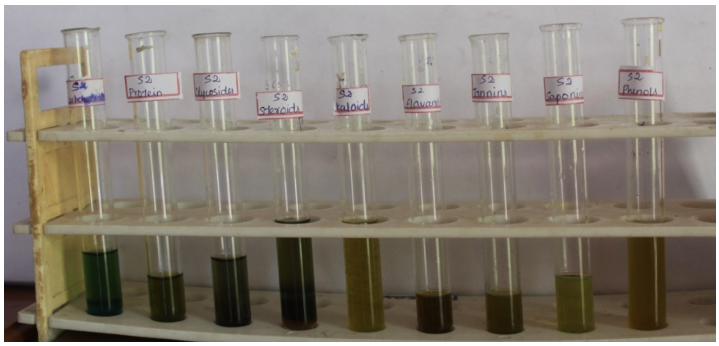


Fig. 1. Visual observation of phytochemical constituents from the plant leaves of *Eleusine indica.*

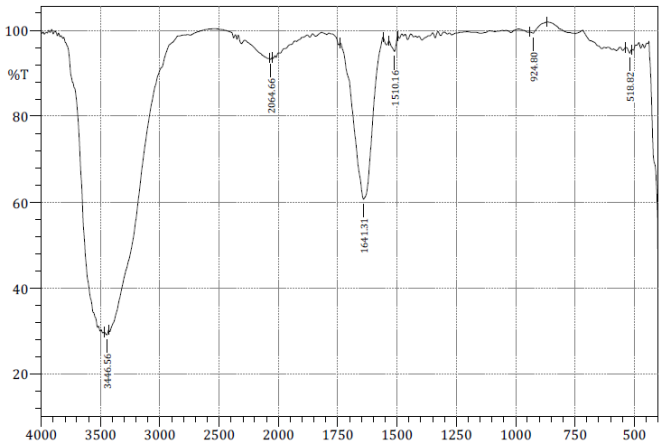


Fig. 2. FT-IR Spectrum of gold nanoparticle synthesized from *Eleusine indica.*

Anti-microbial activity. The crude ethanol extract of *Eleusine indica* was found to active against all bacterial strains. The maximum inhibition of zone (16mm) is noticed against *Pseudomonas aeruginosa.* An average zone of 10-11mm was observed against *Escherichia coli* and *Proteus vulgaris.* These results are in accordance with Bragadeeswaram *et al.* (2010) who also reported the anti-microbial assay of this plant. Crude ethanol extract of *Eleusine indica* shows the highest anti-bacterial activity against *Pseudomonas aeruginosa* (16mm). Hence for the crude ethanol extract of *Eleusine indica*, *Pseudomonas aeruginosa* may be considered the most active bacterial strain as shown in Graph 1 and Fig. 3 respectively.

In vitro Anti-oxidant activities. The most active extracts were scavengers of free radicals and may perform as primary anti-oxidants, and will behave with free radicals by giving hydrogen. The plants obtained from anti-oxidant can reduce the existence of free radicals and will reduce the diseases enhanced by oxidative stress. DPPH radical scavenging activity of the ethanol extract of *Eleusine indica* showed 28.52% (100µg/ml). The crude extract's maximum in-vitro nitric oxide scavenging activity was 40.54% at 100µg concentration. The reason is due to the flavonoids and alkaloids components exhibited from the extracts ledger for the anti-oxidative property of plant leaves. The sustained DPPH level significantly dropped by *E. indica* extract in a dose-dependent manner. The IC50 value, or half maximum inhibitory concentration, was 2350 g/ml. It was observed that the total phenolic content per g of extract was 14.9 0.002 mg/g total phenolic, represented as gallic acid equivalent (Iqbal and Gnanaraj 2012).

Anti-cancer activity. The determination of cytotoxicity was done through MTT Assay. It is found that the most active crude extract of *Eleusine indica* showed the maximum venture against the MCF-7 cell line. The cell viability (%) varied from 91.91% (10µg/ml) to 34.09% (100µg/ml). From the results, the increasing concentration will decrease the cell viability was proven. The active crude extract of *Eleusine indica* showed an IC_{50} concentration value of 85.09% and they are represented in (Table 2, Graph 2 and Fig. 4). The CC50 values for crude extract and hexane fraction were 2.07 and 5.62 mg/ml, respectively, according to cytotoxicity testing against Vero cells using the MTT method (Rashidah Iberahim *et al*., 2015).

Methanolic and hexane extracts *E. indica* was reported as contained the property of anti-proliferative activity against human liver cancer (HepG2) and African green monkey kidney epithelial normal (Vero) cell lines evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Although the IC50 values were calculated as of 91.02 ± 5.74 and 85.30 ± 3.03 and CC50 values of >1000 and 639.39 ± 13.97 g/mL for both extracts. HepG2 cancer cells were more selective than Vero normal cells for SI values of 10.99 and 7.50 (Syahirah Sukor *et al*., 2022).

(Iqbal and Gnanaraj 2012).

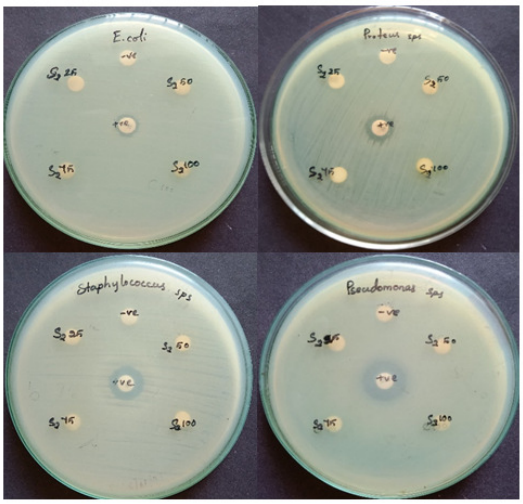


Fig. 3. Antimicrobial activity against *Eleusine indica.*

MCF-7-MTT Assay-*Eleusine indica*

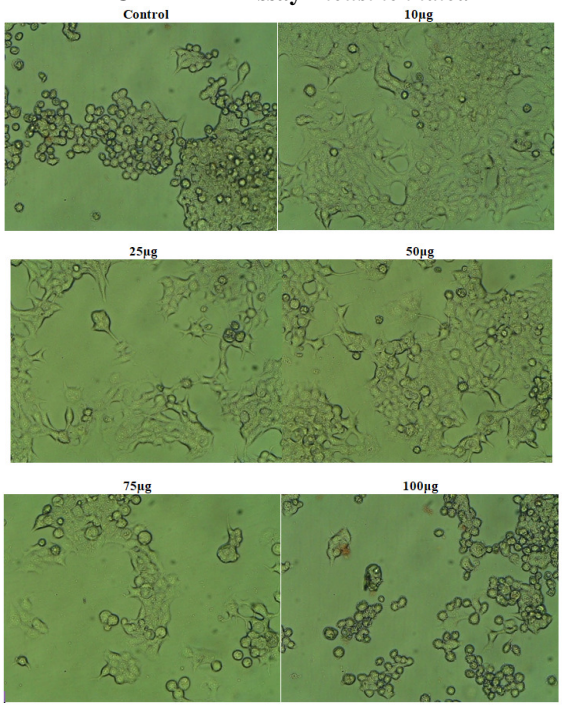
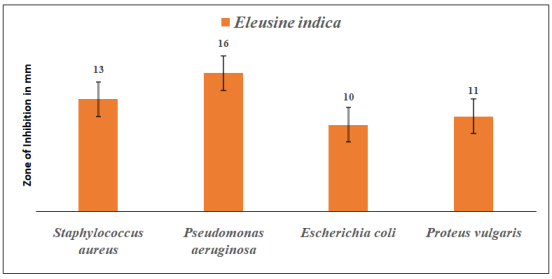
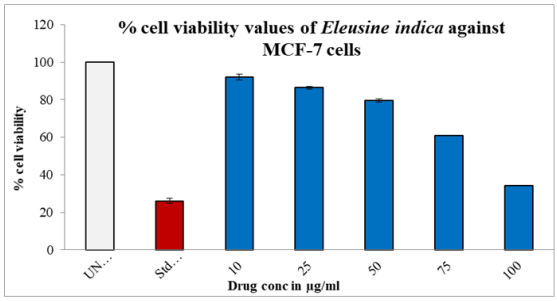


Fig. 4. MTT Assay-Activity of sample-*Eleusine indica.*



Graph 1: Order of antimicrobial activity against *Eleusine indica.*



Graph 2: % cell viability values of (*Eleusine indica*) against MCF-7 cells after the incubation period of 24hrs.

CONCLUSIONS

Medicinal plants are considered as important source of promising bioactive compounds. The quantitative analysis of the leaves of *Eleusine indica* shows the highest steroid content. Phytochemical qualitative analysis was analysed to exhibit the existence of number of secondary metabolites in per capita sample. The preliminary data of the bouncing compounds present in the leaves of *Eleusine indica* was accountable for *in-vitro* norcotic activity. FT-IR Spectra validated the existence of functional groups that exist in secondary metabolites. In the present study, the leaves of *Eleusine indica* contain distinctive secondary metabolites which have anti-oxidative and consanguineous potential. The cytotoxic activity for maxima indicated the polar nature of bouncing components existed in the leaf extract. Overall, the crude extracts, though belong to varied families, they are prospective contender in the province of drug enlargement. Thus, *in-vitro* studies signified that folkaric medicine can be as efficacious as contemporary medicine to battle pathogenic microorganisms. From the current study, manifestation of the efficacy of the plant leaves obtained from conventional healers gave a clear cut-out. These results from the existing study can lay out the outline for refined justification of biologically active components and open on to extra secondary metabolites exploration.

FUTURE SCOPE

In forthcoming, different *in-vitro* and *in-vivo* legitimate studies can be accomplished to probe its biomedical properties. Further examinations will be put forward to analyse its biological activity and trials as clinicals which were necessary for discovery of new drugs formulations.

Acknowledgement. First of all, I thank to God Almighty, for his grace and his presence in all my endeavors. I am thankful to my Research Centre, Holy Cross College (Autonomous), Nagercoil for their constant support and encouragement. The authors are grateful for the technical support rendered by Inbiotics, Nagercoil-03, Kanniyakumari District, Tamilnadu, India and BioHub Research Consultancy for the analysis of FT-IR and biological studies.

Conflict of Interest. None.

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How to cite this article: B. Jone Magadelin, S. Ajith Sinthuja and S. Jameer Ahamed (2023). Phytochemical Screening and Biological activities of *Eleusine indica* leaf extract. *Biological Forum – An International Journal, 15*(3): 444-449.