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PHYTOCHEMICAL SCREENING AND EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF *EULOPHIA NUDA* LIND. TUBER EXTRACTS

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Keywords:

Eulophia nuda, Antibacterial activity, Antifungal activity, Hepatoprotective activity

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ABSTRACT: *Eulophia nuda* Lind. belongs to family Orichidaceae and is a rare and endangered orchid. Present research work was carried out on tuber extracts of *Eulophia nuda* for the evaluation of antimicrobial activities and hepatoprotective. Preliminary phytochemical screening revealed presence of phytochemical constituents like alkaloids, flavonoids, steroids, glycosides (cardiac), tannins, saponins, carbohydrates in three tuber extracts prepared by using solvents (chloroform, acetone and ethanol). Antibacterial activity was carried out with Disc Diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Acetone extract was more effective against *Staphylococcus aureus* with maximum zone of inhibition 18 mm compared to standard antibiotic Ampicillin with zone of inhibition 20 mm. Antifungal studies was carried out using well diffusion method against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. Chloroform extract was more effective against *Aspergillus niger* having zone of inhibition 17 mm compared with standard antifungal Fluconazole (20mm). Acetone extract shown the zone of inhibition of 16 mm against *Aspergillus flavus* compared to standard Fluconazole (22mm). Hepatoprotective activity was carried out as per OECD guidelines 425 using Wistar albino rats. Effect of these extracts on CCl_4 induced hepatotoxic rats was studied by SGOT, SGPT and ALP parameters compared with standard LIV 52. From the research work, it was concluded that *Eulophia nuda* tuber extracts are active as antibacterial, antifungal and hepatoprotective which could be used for the development of some promising formulations, furthermore, structural elucidation of isolated components from the extracts of *Eulophia nuda* can be carried out using studies like IR and NMR.

INTRODUCTION: Traditional herbal medicines are naturally occurring; plant derived substances with minimal or no industrial processing that have been used to treat illness with local or regional healing practices.

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Herbal medicines also known as botanical / phyto-medicine refers to using a plant, seeds, berries, roots, leaves, barks, tubers or flowers for their medicinal purposes [1]. The family Orichidaceae to which orchid belongs is the largest family amongst monocotyledons contains almost 600 - 800 genera / species.

The genus *Eulophia* is terrestrial with almost round pseudo bulbs enveloped by a few sheath carrying 3 - 4 lanceolate, plicate, acuminate, long plicate, long grooved stalks which have several leaf like bracts. The plants blooms in springs with tall thick fleshy

few to several (2-10) followed in inflorescence [2]. The present research work concern primarily with basic extraction procedures for crude drugs to obtain therapeutically desirable portion and eliminate the unwanted material by treatment with a selective solvent, known as menstrum. The principal methods of extraction are maceration, percolation, digestion, infusion, decoction, etc.

The *Eulophia* species is distributed in Eastern Himalaya, from Nepal eastward to Assam Deccan, Kokan southwards, Kerala, Madhya Pradesh, Maharashtra, Sri Lanka, Orissa, Rajasthan, Himachal Pradesh, Tamil Nadu, West Bengal, Uttarakhand, Jharkhand state of India and out of India also. In world, there are almost 230 species of *Eulophia.* In India, around 28 species are recorded all over. Out of these, 20 species have ethanomedicinal uses like analgesic, antipyretic, immunomodulatory, nervine tonic, antidote, anti epileptic, anticancer, anti scrofulous, hepato-protective, aphrodisiac, anti fertility, anti fatigue, vermifuge, health tonic, anti malarial, stomatitis, relieving paralysis, cardio protective, and nephroprotective [3, 4, 5].

MATERIAL AND METHOD:

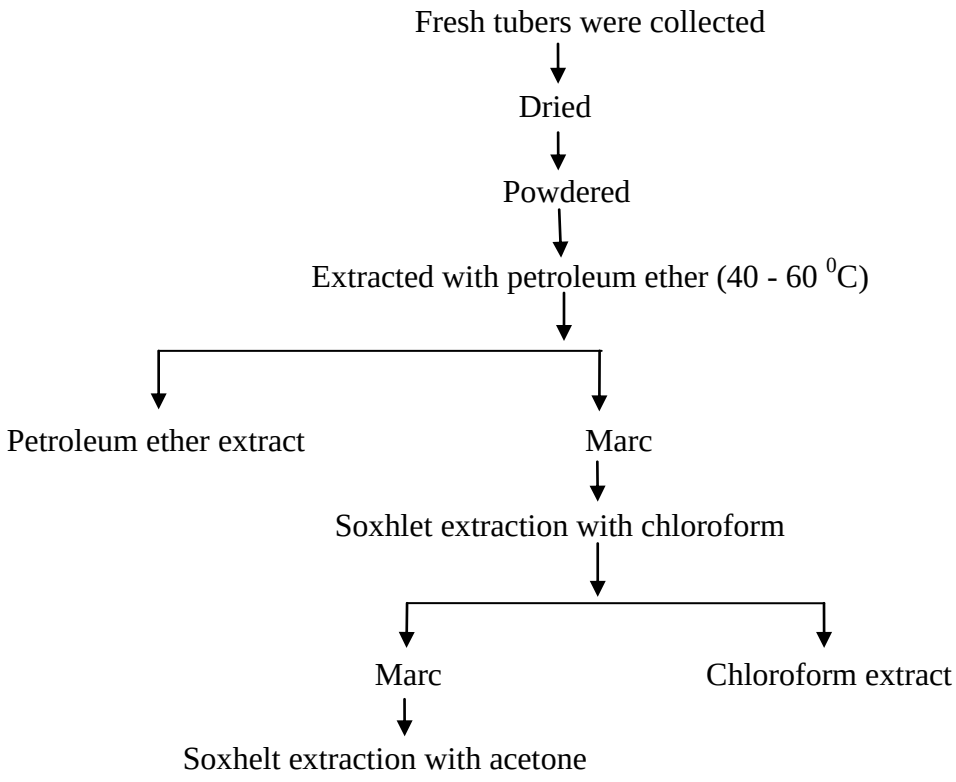
Collection of Kukadkand (Amarkand) Tubers: *Eulophia nuda* tubers were collected randomly during the month of September from Kale Sir (Upavan Society for research and development activity), Nagpur, India.

Authentication of Plant Species: The leaves and tubers of *Eulophia nuda* plant commonly known as "Kukkadkand" was authenticated by Dr. S. N. Malode from Government Vidarbha Institute of Science and Humanities, Amravati, Department of Botany. Reference no. GVISH/BOT Report/12/ 2015.

Taxonomy:

Kingdom:	Plantae
Phylum:	Magnoliphyta
Class:	Liliopsida
Order:	Asparagales
Family:	Orichideaceae
Genus:	*Eulophia*
Species:	*Eulophia nuda*

Solvent: Solvent or extraction agents used in the preparation of phyto-pharmaceuticals must be suitable for dissolving the important therapeutic drug constituents and thus for separating them from the substance containing the drug which are to be extracted.



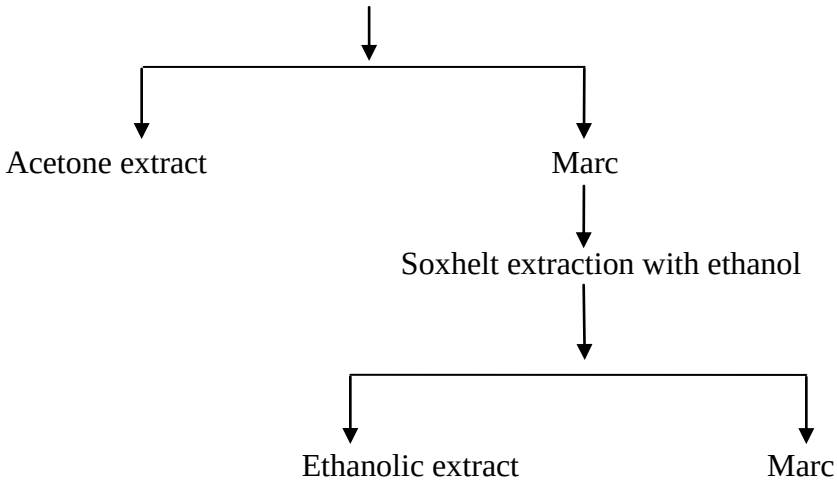


FIG. 1: SCHEMATIC DIAGRAM FOR PROCESS OF EXTRACTION [6]

The extraction was carried out by using different solvents in increasing order of polarity. The following solvents are used for extraction.

1. Petroleum ether (60 - 80 ºC),
2. Chloroform,
3. Acetone,
4. Ethanol.

Preparation of Extract: The dried tubers of *Eulophia nuda* were coarsely powdered, defatted with petroleum ether (40 -60 ºC) and then extracted with the increasing order of polarity of solvents such as chloroform, acetone and ethanol. All the extracts were concentrated under the vacuum and dried.

Preliminary Phytochemicals Screening of Tuber of *Eulophia nuda*: [7, 8] Phytochemical screening of Chloroform extract of *Eulophia nuda* reveals that it contains alkaloids and flavonoid. Alcoholic extracts revealed that it contains Flavonoid, cardiac glycoside, Saponin, alkaloid, and, tannin and acetone extract contain cardiac glycoside, Saponin and alkaloid. Phytochemical screening of Chloroform extract of *Eulophia nuda* reveals that it contains alkaloids and flavonoid. Alcoholic extracts revealed that it contains Flavonoid, cardiac glycoside, Saponin, alkaloid, and, tannin and acetone extract contain cardiac glycoside, Saponin and alkaloids. TLC was carried out by using Ethanol extracts, chloroform extracts and acetone extracts of *Eulophia nuda* tubers [9, 10].

Preliminary Phytochemical Screening of *Eulophia nuda:*

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF *EULOPHIA NUDA*

Sr. no.	Plant Constituents	Test/ Reagents	Ethyl alcohol	Chloroform	Acetone
1	Alkaloids	Modified dragandroff's	+	-	-
		Hager's reagent	+	-	-
		Wagner's reagent	+	+	+
		Mayer's reagent	+	+	+
		Dragandroff's reagent	+	+	-
2	Flavonoid	Shinoda test	+	+	-
		Alkali test	+	-	-
		Lead test	+	-	-
3	Steroid	Salkowaski reaction	+	-	-
		Liebermann - Burchard test	+	-	-
4	Tannins	Ferric chloride test	+	+	-
		Iodine test	+	+	-
5	Saponin	Foam test	+	+	+
6	Cardiac glycoside	Baljet test	+	+	+
		Legal test	+	-	+
		Killer Killani Test	+	-	+

Note: (+) Indicate present and (–) Indicate absence.

Pharmacological Activities:

Antibacterial Activity: Antibacterial activity of *Eulophia nuda* was carried out by using test organism (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) with disc diffusion method. Extracts were used at concentration of 1mg/ml along with standard antibiotic Ampicillin. Test organisms was maintained at nutrient medium (Sabouraud dextrose agar medium) which was sterilized by autoclaving at 151 b/sq. inch pressure for 30 mins.

Preparation of Standard Drug: Ampicillin (1mg/ ml) in DMSO solution. All extracts were dissolved in DMSO separately to get 10mg/ml solution.

Method:

Disc Diffusion Assay: Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of antibacterial activity in extract. 1ml of culture was placed in Petri plate and 20ml of sabouraud agar was poured. The two were mixed thoroughly. The plates were allowed to solidify and dry for 15 minutes before use in the test. Wells were then created and a pipette was used to place 50µl of diluted extract into each well. The plates were incubated at 37 ºC and 28 ºC for 3 - 5 days after which they were examined for inhibition zones. Calibrated plastic scale was used to measure the inhibition zones. The test experiment was repeated two times to ensure reliability [11, 12].

Antifungal Activity: Antifungal activity of *Eulophia nuda* tuber extracts was carried out by using test organism (*Aspergillus niger, Aspergillus flavus, Candida albicans*) with well diffusion method. Extracts were used at concentration of 1 mg/ml along with standard antifungal Fluconazole. Test organisms was maintained at nutrient medium (Sabouraud dextrose agar medium) which was sterilized by autoclaving at 151 b/sq. inch pressure for 30 mins.

Preparation of Standard Drug: Fluconazole (1mg/ml) in DMSO solution. All extracts were dissolved in DMSO separately to get 10mg/ml solution.

Method:

Well Diffusion Assay: Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of antifungal activity in extract. The plates were allowed to solidify and dry for 15 minutes before use in the test. Wells were then created and a pipette was used to place 50µl of diluted extract into each well. The plates were incubated at 37 ºC and 28 ºC for 3-5 days after which they were examined for inhibition zones. Calibrated plastic scale was used to measure the inhibition zones. The test experiment was repeated two times to ensure reliability [13].

Hepatoprotective Activity: [14] Hepatoprotective activity was carried out by acute toxicity study.

Acute Toxicity: Acute toxicity study of successive extracts of acetone, chloroform, and ethanol were performed as per OECD guidelines 425 using Wistar albino rats. Animals were fasted 4 h before receiving the test extracts. Acetone, chloroform, ethanol extracts were given orally at dose of 2000 mg/kg body weight suspended in 1% CMC. For each extract, 5 animals were used. After dosing, animals were observed individually once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 hours, and daily thereafter, for a total period of 14 days. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose.

If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000mg / kg body weight.

Carbon tetrachloride (CCl_4) Induced Hepatotoxicity: The successive extracts of *Eulophia nuda* were evaluated for Hepatoprotective activity in acute CCl_4 induced hepatotoxicity in rats. The Wistar albino rats (180-240 g) were divided into 11 groups as mentioned below (n=5). The group 1 animals, served as normal control and received only vehicle (1% CMC), while the animals of group 2 served as toxic control. Animals of group 3 were treated with Liv52 at a dose of 5ml/kg as standard drug for 7 days. Animals of group 4 to 11 were treated with successive extracts of *Eulophia nuda* 7 days as shown in **Table 3**.

On the seventh day, all animal groups except for control received an intraperitoneal dose of carbon tetrachloride (CCl_4) at 1 ml/kg body weight (1:1 in olive oil), 30 min after the respective treatment with extracts, Liv52 and vehicle. *Eulophia nuda*

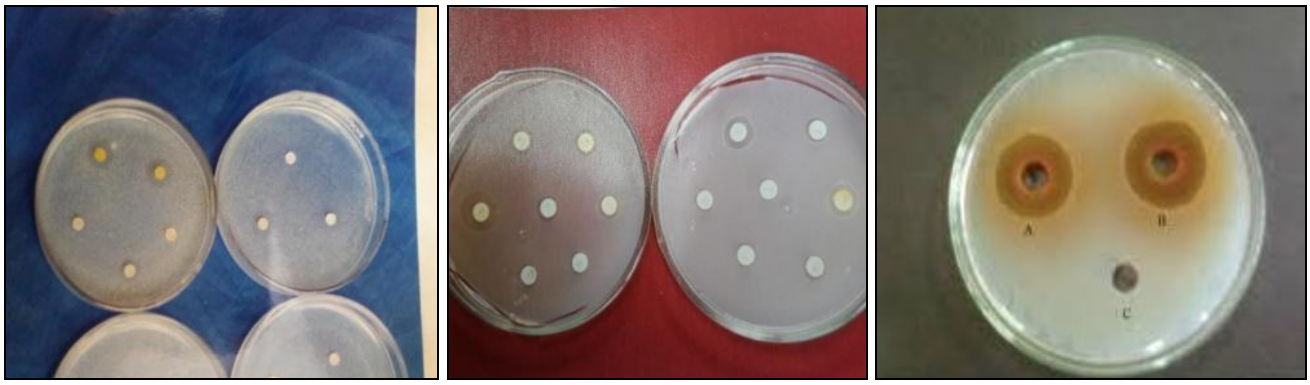
extracts and standard Liv52 suspended in 1% CMC were administered orally for 7 days.

a) By using various extracts (ethanol, chloroform, and acetone) of dried powder of *Eulophia nuda* tubers.

b) By using slurry of dried powder of *Eulophia nuda* tubers.

RESULTS:

Antibacterial Activity: Antibacterial activity was carried out with Disc Diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Acetone extract was more effective against *Staphylococcus aureus* with maximum zone of inhibition 18 mm compared to standard antibiotic Ampicillin with zone of inhibition 20 mm.



Escherichia coli ***Pseudomonas aeruginosa*** ***Staphylococcus aureus***

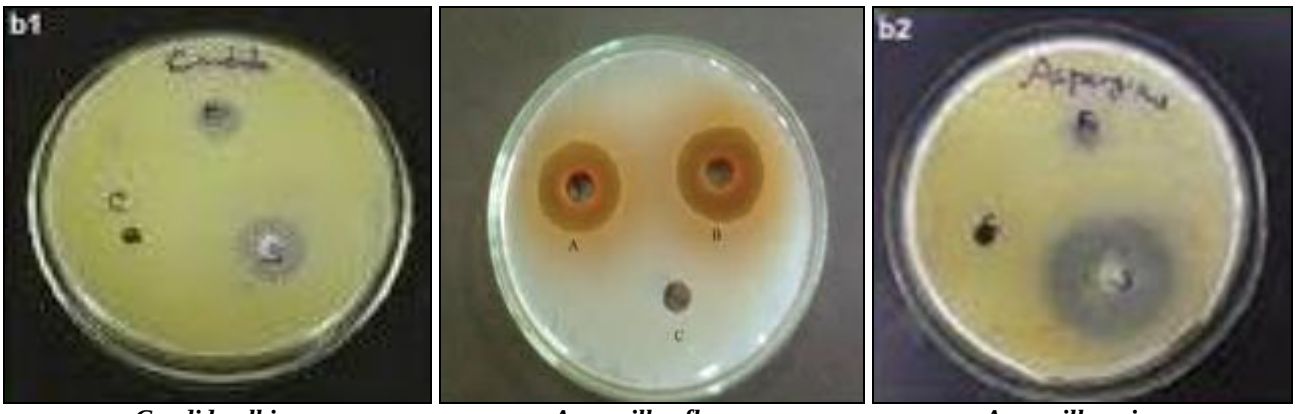
FIG. 2: ANTIBACTERIAL ACTIVITY

TABLE 2: RESULTS OF ANTIBACTERIAL ACTIVITY

		Zone of inhibition in mm		
S.no.	**Extracts of *Eulophia nuda***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
1	Alcoholic extract	11	9	12
2	Chloroform extract	10	R	14
3	Acetone extract	12	14	18
4	Ampicillin (5mg/ml)	16	20	20

R- Resistant (no zone of inhibition)

Antifungal Activity: Antifungal studies were carried out using well diffusion method against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. Chloroform extract was more effective against *Aspergillus niger* having zone of inhibition 17mm compared with standard antifungal Fluconazole (20mm). Acetone extract shown the zone of inhibition of 16 mm against *Aspergillus flavus* compared to standard Fluconazole (22 mm).



Candida albicans ***Aspergillus flavus*** ***Aspergillus niger***

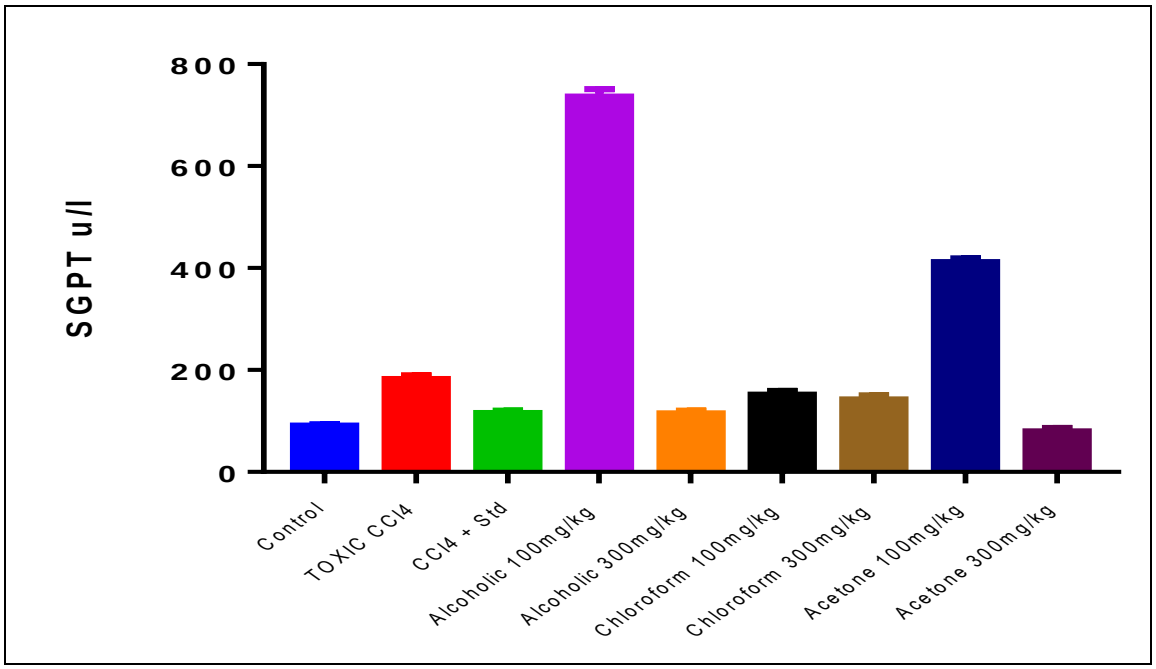
FIG. 3: ANTIFUNGAL ACTIVITY

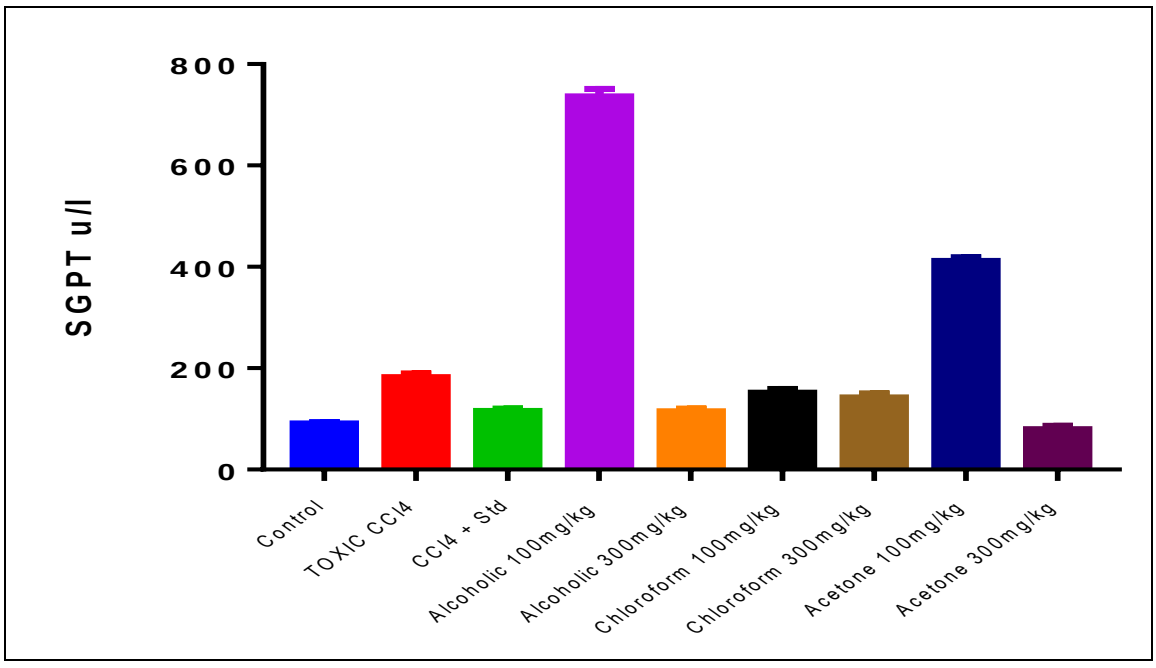
TABLE 3: RESULTS OF ANTI FUNGAL ACTIVITY

S. no	Tuber extract from *Eulophia nuda*	Zone of inhibition in mm		
		Candida albicans	*Aspergillus niger*	*Aspergillus flavus*
1	Alcohol	12	16	8
2	Chloroform	10	17	R
3	Acetone	R	R	16
4	Fluconazole (1mg/l)	18	20	22

8-12 mm - Moderately sensitive, >12 mm- Sensitive, R- Resistant

Hepatoprotective Activity: Acute toxicity study was carried out using three successive tuber extracts of acetone; chloroform and ethanol as per OECD guidelines 425 using Wistar albino rats. Effect of these extracts on CCl_4 induced hepatotoxic rats was studied by using SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Peroxides Transaminase), and ALP (Alkaline Phosphates) parameters compared with standard LIV 52.





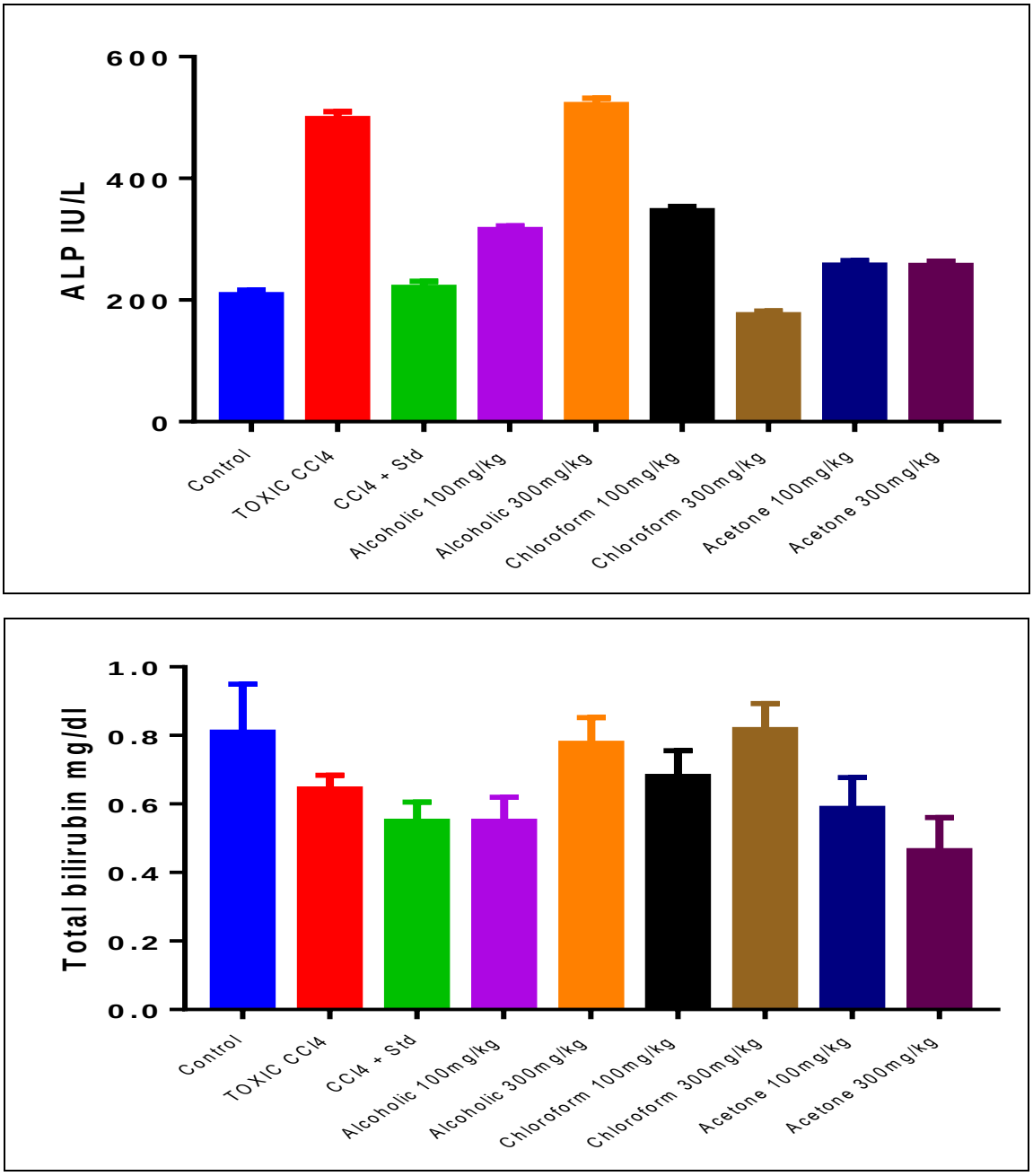


FIG. 4: DIFFERENT GRAPHS SHOWS HEPATOPROTECTIVE ACTIVITIES

TABLE 4: LEVEL OF SGOT (SERUM GLUTAMATE OXALOACETATE TRANSAMINASE), SGPT (SERUM GLUTAMATE PEROXIDES TRANSAMINASE), ALP (ALKALINE PHOSPHATES), TOTAL BILIRUBIN

Group No.	Groups	SGOT	SGPT	ALP	Total Bilirubin
I	Normal	137u/L	94u/l	211u/l	0.71md/dl
II	Control(CCl_4)	171u/l	181u/l	498u/l	0.68mg/dl
III	Standard LIV 52	164u/l	116u/l	222u/l	0.56mg/dl
IV	Ethanol extract 100mg/kg	865u/l	738u/l	315u/l	0.5mg/dl
V	Ethanol extract 300mg/kg	185u/l	110u/l	524u/l	0.78Mg/dl
VI	Chloroform extract 100mg/kg	186u/l	150u/l	346u/l	0.69mg/dl
VII	Chloroform extract 300mg/kg	191u/l	141u/l	170u/l	0.80mg/dl
VIII	Acetone extract 100mg/kg	405u/l	335u/l	257u/l	0.58mg/dl
IX	Acetone extract 300mg/kg	212u/I	73u/l	256u/l	0.44mg/dl

DISCUSSION: Present research work is an attempt to evaluation of pharmacological activities such as Hepatoprotective, antibacterial, antifungal etc of the extracts of *Eulophia nuda*. The plant has

not been explored for its Hepatoprotective activity. The literature survey reveals that the *Eulophia nuda* contains alkaloid, tannins, Saponin, Flavonoid, cardiac glycoside, steroid which is known to possess antioxidant, cytotoxic activity. TLC was carried on ethanolic extract, chloroform extract and acetone extract.

Antibacterial study was done by using Disc diffusion method. The Acetone extract was resistant against *Escherichia coli*, *Pseudomonas aeruginosa.* Acetone extract is more effective against *Pseudomonas aeruginosa* having maximum zone of inhibition 18 mm. Antifungal study was done by using well diffusion method. It reveals that zone of inhibition chloroform extract had moderate antifungal activity but quite good than ethanol and acetone extract. The result of hepatoprotective activity reveals that the acetone extract was less effective in hepatotoxic rats than alcoholic and chloroform extract.

CONCLUSION: In the present research work, phytochemical screening and evaluation of pharmacological activities like antimicrobial and hepatoprotective of *Eulophia nuda* tuber extracts revealed that the tuber is active as hepatoprotective when compared with standard LIV 52 on Wistar albino rats. The extracts were also found to be effective as antibacterial against *Escherichia Coli*, *Pseudomonas aeruginosa and Staphylococcus aureus* using standard ampicillin and as antifungal against *Candida albicans, Aspergillus flavus and Aspergillus niger* using the standard fluconazole.

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CONFLICTS OF INTEREST: Nil

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