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## **PHYTOCHEMICAL SCREENING AND EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF *BUCHANANIA LANZAN* SPRENG LEAVES**

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**ABSTRACT:** The aim of this research was to perform phytochemical screening and evaluation of the pharmacological activities of *Buchanania lanzan* Spreng. leaves. Preliminary phytochemical screening revealed the presence of phytoconstituents like steroids, tannins, saponins, carbohydrates, alkaloids, and flavonoids. Three leaf extracts were prepared by using solvents e.g. chloroform, ethyl acetate, and ethanol. The antibacterial activity was evaluated by using the paper disc method against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The chloroform extract was found to be more effective against *Pseudomonas aeruginosa* with a zone of inhibition 20 mm as compared to the standard anti-biotic ampicillin with a zone of inhibition 20 mm. Antifungal activities were evaluated by well diffusion assay against *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger*. The chloroform extract was found to be more effective against *Aspergillus flavus* and *Candida albicans* with a zone of inhibition 22 mm and 20 mm respectively as compared to the standard antifungal ketoconazole with a zone of inhibition 22 mm. Antifungal activity against *Aspergillus niger* with zone of inhibition 22 mm was comparable to standard Ketoconazole with zone of inhibition 24 mm. Anthelmintic activity was performed on *Pheritima posthuma* using Albendazole as standard and ethanolic extract was found to be more effective. Hepatoprotective activity performed on Wistar albino rats revealed that ethyl acetate extract was more promising using LIV 52 as standard. Therefore, it can be concluded that the *Buchanania Lanzen* leaves specific extracts possess antimicrobial, anthelmintic and hepatoprotective activity which can be further explored for the development of formulation and their structural elucidation.

**INTRODUCTION:** *Buchanania lanzan* Spreng. a member of family Anacardiaceae is a commercially useful tree, first described by Francis Hamilton in 1798 **Fig. 1**. It is commonly known as char, achar or chironji. The tree is native in the tropical deciduous forests of Northern, Western and Central India.

It is also found in other tropical Asian countries, Australia and the Pacific islands <sup>1</sup>. The tree grows well on yellow sandy-loamy soil. The bark of *Buchanania lanzan* Spreng. is rough, with dark grey or black color. Leaves are broadly oblong, have rounded base with a blunt tip and straight parallel veins. The flowering period is between January to March. Flowers are small, greenish-white.

The fruit is a yellowish-red drupe, one-seeded, which ripens between April to May <sup>2, 3</sup>. Mehta *et al.*, have reported the presence of glycosides, phenolic compounds and flavonoids in the different leaf extracts of *Buchanania lanzan* from



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preliminary phytochemical analysis<sup>4</sup>. Khatoon *et al.*, have reported the nutraceutical and phytochemical screening and demonstrated that *Buchanania lanza* is a very rich source of polyphenols, flavonoids, tannins, alkaloids and saponins<sup>5</sup>. Seeds were reported to be rich sources of phosphorus, calcium, magnesium, iron, tocopherols, and essential fatty acids like oleic acids, linoleic acid, and linolenic acid. The phytochemical analysis reported by Pattnaik *et al.*, revealed the presence of flavonoids, tannins, glycosides, phenols, steroid, saponin, gallic acid and myricetin 3'-rhamnoside-3-galactoside in the leaves<sup>6</sup>. In 2017, Joshi *et al.*,<sup>7</sup> have reported the anti-depressant activity of hydroalcoholic extract of *Buchanania lanza* fruits. Kodati *et al.*,<sup>8, 9</sup> have reported anti-diarrhoeal and anti-ulcer activity of ethanolic extract of *Buchanania lanza* Spreng. roots. Tyagi *et al.*,<sup>10</sup> reported *in-vivo* diuretic and anti-ulcer activity in fruits of *Buchanania lanza*. Methanolic extract of roots of *Buchanania lanza* Spreng. Were also reported to possess analgesic and anti-inflammatory activities<sup>11</sup>.



**FIG. 1: BUCHANANIA LANZA SPRENG PLANT**

The essential oil obtained from seeds of *Buchanania lanza* Spreng. was reported to possess antifungal activity<sup>12</sup>. The leaves of *Buchanania lanza* are also reported to possess several pharmacological activities. In 2011, methanolic extract of leaves of *Buchanania lanza* was reported to possess adaptogenic activity in an experimental study in rat model<sup>13</sup>. Anti-diabetic, anti-hyperlipidemic and antioxidant activity of the methanol extract of leaves of *Buchanania lanza* was reported by Sushma *et al.*, in streptozotocin-induced types I and II diabetic rats<sup>14</sup>. As literature survey suggested the presence of variety of phytoconstituents like steroids, tannins, saponins, carbohydrates, alkaloids, and flavonoids in *Buchanania lanza*, a need was felt to evaluate the plant for its pharmacological activities. Therefore,

the present study was carried out with the aim to perform phytochemical screening and evaluation of antibacterial, anti-fungal, anthelmintic and hepatoprotective activities of *Buchanania lanza* Spreng. leaves.

## MATERIALS AND METHODS:

**Materials:** The leaves of *Buchanania lanza* were collected from a local area of Manasayurved, Nagpur, Maharashtra, India, in September 2015. A solvent such as petroleum ether, chloroform, ethyl acetate, ethanol was purchased from Loba Chemie Pvt. Ltd. Mumbai.

### Methods:

#### **Authentication of *Buchanania lanza* Spreng.**

**Plant:** The plant *Buchanania lanza* was authenticated by Dr. S. N. Malode, Head of Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati. The plant has Reference no. GVISH/BOT/Report/07/2015. A sample voucher specimen of the plant was deposited for future reference.

**Extraction:** The leaves of *Buchanania lanza* was collected and dried in the shade and then pulverized in a grinder. The material was then passed through #120 mesh to obtain uniform size powder. The powdered plant material was successively extracted separately with solvents of increasing polarity like petroleum ether, chloroform, ethyl acetate and ethanol by Soxhlet extractor.

Before extraction with each solvent, the powdered material, as well as each extract (residue), was air-dried below 50 °C, and The completion of extraction was indicated by taking a sample out of siphon tube on TLC plate and placing it in iodine chamber. All the extracts were concentrated under the vacuum, and dried along solvent was distilled off.

**Phytochemical Screening:** The plant may be considered as the biosynthetic laboratory for a multitude of components like alkaloids, glycoside, volatile oils, tannins, and flavonoids. They are termed as secondary metabolites, which are mainly responsible for therapeutic effects. Various extracts of *Buchanania lanza* were subjected to phytochemical screening **Table 1** for identification of different constituents and plant metabolites present in the extract<sup>1</sup>.

**TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF BUCHANANIA LANZAN**

Constituents	Test/Reagents	<i>Buchanania lanza</i> extract		
		Chloroform	Ethyl acetate	Ethyl Alcohol
Alkaloids	Hager's reagent	+	-	+
	Wagner's reagent	-	-	-
	Mayer's reagent	-	-	-
	Dragendorff's reagent	-	-	-
Amino acid	Ninhydrin Test	-	-	-
	Molisch's test	+	+	+
Carbohydrates	Bradford' test	+	+	+
	Biuret test			
	Million's test	-	-	-
Proteins	Xanthoprotein test			
	Shinoda test	+	+	+
Flavonoid	Salkowski reaction	+	+	+
	Liebermann Burchard test	+	+	+
Tannins	Ferric chloride test	+	+	+
	Lead acetate Test	-	-	-
	Nitric acid test	-	+	+
	Gelatin solution test	+	+	-
Saponin	Foam test			+

\*(-) indicates absent, (+) indicates present

**Evaluation of Pharmacological Activity:**

**Antibacterial Activity:**<sup>15-17</sup> Anti-bacterial activity of *Buchanania lanza* was evaluated on *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using paper disc method. Extracts were used at a concentration of 1 mg/ml along with standard antibiotic Ampicillin (1 mg/ml) in dimethyl sulfoxide (DMSO) solution. Test organisms were maintained at nutrient medium (Sabouraud dextrose agar medium) which was sterilized by autoclaving at 151 b/sq. inch pressure for 30 min. All extracts were dissolved in DMSO separately to get 10 mg/ml solution. The nutrient agar plates were prepared by pouring 20 ml of molten media into sterile petriplates under aseptic conditions.

The plates were allowed to solidify for 5 min and 0.5 ml of test culture was inoculated and uniformly spread over the agar surface with the help of a sterile L - shaped bent glass rod. After inoculation, wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well, different extract solution was added separately and controlled were the same maintained. The treated plats were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the discs were measured and diameter was calculated in millimeter.

**Anti-fungal Activity:**<sup>18</sup> Anti-fungal activities of *Buchanania lanza* leaves extracts was evaluated on *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* with well diffusion method. Extracts were used at a concentration of 1 mg/ml along with standard antifungal Ketoconazole. Test organisms were maintained at nutrient medium (Sabouraud dextrose agar medium) which was sterilized by autoclaving at 151 b/sq. inch pressure for 30 min. Ketoconazole (1 mg/ml) in DMSO solution. All extracts were dissolved in dimethyl sulfoxide (DMSO) separately to get 10 mg/ml solution. 1 ml of culture mixed thoroughly. The plates were allowed to solidify and dry for 15 min before use in the test. Wells were then created and a pipette was used to place 50 µl of diluted extract into each well. The plates were incubated at 37 °C and 28 °C for 3-5 days after which they were examined for inhibition zones. The calibrated plastic scale was used to measure the inhibition zones. The test experiment was repeated two times to ensure reliability.

**Anthelmintic Activity:**<sup>19, 20</sup> The anthelmintic activity was evaluated on Indian earthworm *Pheritim posthuma*. The extracts were prepared at a concentration of 10 mg/ml, along with albendazole as a standard anthelmintic. Albendazole (10 mg/ml) prepared in distilled water. The various concentrations (25, 50 mg/ml)

of each extract were prepared in distilled water. Five groups each containing four earthworms of approximately equal size was released into 10 ml of the desired formulation. Each group was treated with one of the following, vehicles (5% DMF in normal saline), albendazole, alcoholic extract, chloroform extract, ethyl acetate extract of leaves of *Buchanania lanza* (25, 50 mg/ml each) in normal saline containing 5% DMF. The living and viable worms were kept under close observation and the time taken to complete paralysis and death were recorded. The motionless worms were transferred to warm water at 40 °C to confirm that they are dead.

**Hepatoprotective Activity:** <sup>21-23</sup> Hepatoprotective activity was carried out by an acute toxicity study. Acute toxicity study of successive extracts of chloroform, ethanol and ethyl acetate was performed as per OECD guidelines 423 using Wistar albino rats. The animals were randomly selected and marked to permit individual

identification. They were kept in the cage five days before dosing to allow for acclimatization to laboratory conditions. The extract of *Buchanania lanza* had been given in various doses (100 - 500 mg/kg) by the oral route. After administration of the extract, the animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, with special attention giving during first 4 h, and duly thereafter, for a total 14 days. Data for mortality at different dose levels were recorded.

## RESULTS:

**Anti-bacterial Activity:** Anti-bacterial activity was carried out with the Disc Diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* **Fig. 2**. The chloroform extract of leaves was more effective against *Pseudomonas aeruginosa* with a maximum zone of inhibition 20 mm as compared to standard antibiotic ampicillin (20 mm) **Table 2**.

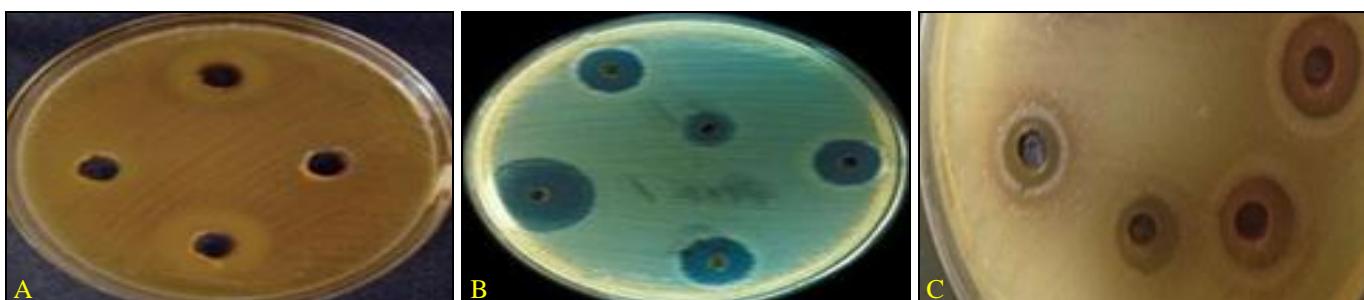


**FIG. 2: ANTI-BACTERIAL ACTIVITY AGAINST A) *ESCHERICHIA COLI* B) *PSEUDOMONAS AERUGINOSA*, C) *STAPHYLOCOCCUS AUREUS***

**TABLE 2: ANTI-BACTERIAL ACTIVITY OF *BUCHANANIA LANZA* LEAVES EXTRACT**

Extracts	Zone of inhibition in mm		
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Alcoholic extract	12	10	R
Chloroform extract	10	20	9
Ethyl acetate extract	R	R	8
Ampicillin (5 mg/ml)	16	20	20

\*R- Resistant (no zone of inhibition)



**FIG. 3: ANTI-FUNGAL ACTIVITY AGAINST A) *CANDIDA ALBICANS*, B) *ASPERGILLUS NIGER*, C) *ASPERGILLUS FLAVUS***

**TABLE 3: ANTI-FUNGAL ACTIVITY OF BUCHANANIA LANZAN LEAVES EXTRACT**

Extracts	Zone of inhibition (mm)		
	Candida albicans	Aspergillus flavus	Aspergillus niger
Alcoholic extract	14	12	12
Chloroform extract	20	22	22
Ethyl acetate extract	R	8	8
Ketoconazole (1mg/ml)	22	22	24

\*R- Resistant (no zone of inhibition)

**Anti-fungal Activity:** Antifungal studies were carried out using well diffusion method against *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* **Fig. 3**. The chloroform extract was found to be more effective against *Aspergillus niger* with a zone of inhibition 22 mm compared with standard anti-fungal ketoconazole (22 mm) as shown in **Table 3**. Acetone extract has shown the

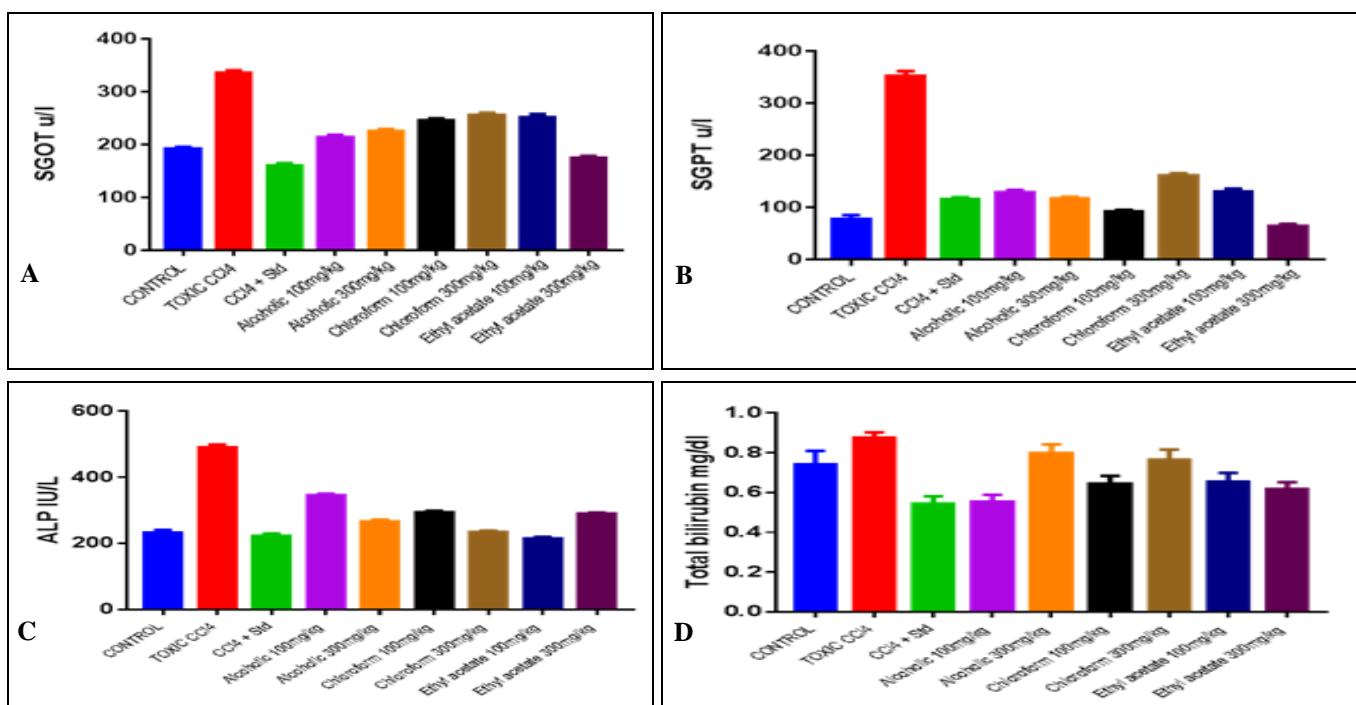
zone of inhibition of 22 mm against *Aspergillus flavus* compared to standard ketoconazole (22 mm).

**Hepatoprotective Activity:** Hepatoprotective activity was evaluated by acute toxicity study as per OECD guidelines 425 using Wistar albino rats using three successive extracts of acetone, chloroform and ethanol were performed **Table 4**. The effect of these extracts on serum cholesterol on CCL<sub>4</sub> in hepatotoxicity in rats was studied by using SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Peroxides Transaminase), and ALP (Alkaline Phosphates) compared with standard Liv 52 **Fig. 4**. The evaluation of hepatoprotective activity revealed that acetone extract was less effective than other extracts in hepatotoxic rats.

**TABLE 4: EFFECT OF VARIOUS EXTRACTS OF BUCHANANIA LANZAN LEAVES (CHLOROFORM, ETHYL ACETATE AND ALCOHOL) ON SGOT (U/L), SGPT (U/L), ALP (IU/L) AND BILIRUBIN (MG/DL) IN CCl<sub>4</sub> INDUCED LIVER DAMAGE**

Group	Treatment	SGOT (U/L)	SGP (U/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
I	Control	192.3 ± 3.05	78 ± 7.5	232.7 ± 7.5	0.74 ± 0.07
II	Toxic	335.3 ± 5.03	352 ± 9.1	489.0 ± 9.0	0.88 ± 0.025
III	CCl <sub>4</sub> + Std	159.3 ± 5.03	115 ± 4.5	222.0 ± 7.0	0.5433 ± 0.04
IV	Alcoholic extract 100 mg/kg	214.3 ± 4.04	129.3 ± 4.04	344.7 ± 5.0	0.5533 ± 0.035
IV	Alcoholic extract 300 mg/kg	224.7 ± 4.50	117.7 ± 2.5	265.7 ± 6.0	0.7967 ± 0.045
VI	Chloroform extract 100 mg/kg	245.0 ± 4.35	91.33 ± 4.04	294.3 ± 4.04	0.6433 ± 0.04
VII	Chloroform extract 300 mg/kg	255 ± 5.0	161.3 ± 4.16	234.3 ± 4.0	0.7667 ± 0.05
VIII	Ethyl acetate 100 mg/kg	251.3 ± 6.5	130.7 ± 5.50	214.7 ± 5.03	0.6533 ± 0.045
IX	Ethyl acetate 300 mg/kg	174.3 ± 4.04	64.33 ± 4.04	290.3 ± 2.5	0.6167 ± 0.035

Student's t-test was performed. Each value represents the mean ± S.E.M. of 5 rats in each group. P values < 0.05 as compared with group II (CCl<sub>4</sub> treated group)

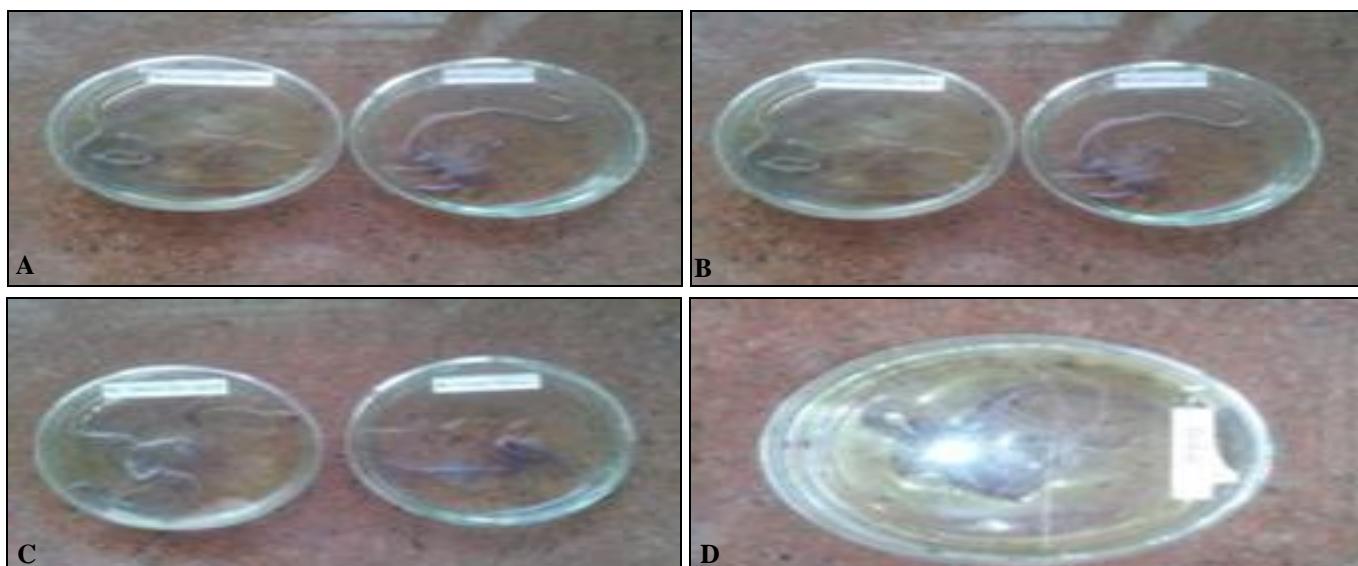
**FIG. 4: LEVEL OF A) SGOT, B) SGPT, C) ALP, D) BILIRUBIN IN GROUPS I-IX IN CCl4 INDUCED LIVER DAMAGE**

**Anthelmintic Activity:** Anthelmintic activity **Fig. 5** showed that alcoholic extract was more effective

as it takes less time for paralysis and death of earthworms in **Table 5**.

**TABLE 5: EFFECT OF *B. LANZAN* EXTRACT OF VARIOUS CONCENTRATIONS ON EARTHWORM**

Test Substance	Concentration (mg/ml)	Time is taken by <i>Pheretima Posthuma</i> for paralysis (P) and Death (D) of worms in minutes	
		P	D
Alcoholic extract	25	27	40
	50	18	34
Chloroform extract	25	38	53
	50	28	45
Ethyl acetate extract	25	30	52
	50	20	40
Standard Albendazole	25	14	28



**FIG. 5: EARTHWORMS IN A) ALCOHOLIC EXTRACT, B) ETHYL ACETATE EXTRACT, C) CHLOROFORM EXTRACT, D) ALBENDAZOLE**

**DISCUSSION:** Present research work aimed to evaluate potential hepatoprotective, antibacterial, antifungal and anthelmintic activity of extracts of *Buchanania lanzan* leaves. As per our knowledge, the plant has not been explored for its hepatoprotective activity to date. The literature survey reveals that the *Buchanania lanzan* contains tannins, saponins, flavonoids, carbohydrates which are known to possess anti-oxidant, antimicrobial activity. The anti-bacterial study was performed using disc diffusion method. The zone of inhibition against various bacterial strains is shown in **Table 2**. The results reveal that chloroform extract is more effective against *Pseudomonas aeruginosa* with maximum zone of inhibition 20 mm. Alcoholic extract was resistant to *Staphylococcus aureus* while ethyl acetate extract was resistant to *Escherichia coli* and *Pseudomonas aeruginosa*. The results of anti-fungal activity are shown in **Table 3**. It reveals that chloroform extract and alcoholic extract had antifungal activity against all

fungal strains. However, ethyl acetate extract was found to be less active as anti-fungal. Assessment of hepatoprotective activity, the degree of hepatotoxicity developed was determined by withdrawing blood and evaluating different parameters on seventh day. The elevated level of SGOT, SGPT, ALP, and total bilirubin indicates the hepatotoxicity. According to observed biochemical parameters obtained **Table 4**, ethyl acetate extract showed a marked reduction in elevated enzyme level, and it shows activity like Liv52 standard drug **Fig. 4**. It can be concluded that ethyl acetate extract was effective in hepatotoxicity. The anthelmintic study was performed on Indian earthworm *Pheritima posthuma* using Albendazole as a standard drug. According to results showed in **Table 5**, the alcoholic extract showed more anthelmintic activity as compared to ethyl acetate and chloroform extracts.

**CONCLUSION:** The antibacterial, antifungal, the anthelmintic and hepatoprotective activity of *Buchanania lanza* leaves extract was evaluated. The ethyl acetate extract of *Buchanania lanza* leaves was found to be active as a hepatoprotective. The plant exhibited an antibacterial effect against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Antifungal effect against *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger*. Moreover, the leaves extract also exhibited moderate anthelmintic activity. Therefore, it can be concluded that *Buchanania lanza* leaves possess hepatoprotective, anti-bacterial, anti-fungal and anthelmintic activity.

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**CONFLICTS OF INTEREST:** The authors declare no conflict of interest.

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