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***Research Article** (Open Access)*

EVALUATION OF ETHNOVETERINARY PROPERTIES OF *CARDIOSPERMUM HELICACABUM, ANDROGRAPHIS PANICULATA* AND *TRIBULUS TERRESTRIS* AGAINST BACTERIAL ABSCESS OF *FELIS CATUS*

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ABSTRACT
Bacterial abscess in domestic cat (*Felis catus*) is a localized infection caused by bacteria resulting in the formation of a pocket of pus surrounded by inflamed tissue occurring in any part of the body, commonly found on the skin. The infection usually develops after skin has been punctured, allowing bacteria to enter the wound and proliferate, which results in redness, swelling, pain, and the presence of a lump under the skin. Proper and timely treatment for bacterial abscess in animals is thus important as it can lead to serious complications, if left untreated. Modern veterinary practices are however unavailable or unaffordable in many remote areas where domestic animals are plenty in number. The rural population still follow traditional veterinary practices, deep rooted in culture that involve use of medicinal plants and other natural remedies to prevent and treat animal diseases, referred to as Ethnoveterinary medical practices. In this study, swab samples of abscess of *Felis catus* were collected and the isolated bacterial strains were inoculated on an agar plate to screen the inhibitory potentials of selected medicinal plant extracts against the pathogens isolated. Simultaneously, leaf extracts of *Cardiospermum helicacabum, Andrographis paniculata* and *Tribulus terrestris* were prepared and analyzed for the presence of phytochemicals. Various concentrations of the plant extracts were used for the screening of their inhibitory potentials against the pathogens and the results showed significant antibacterial activities. These findings suggest that traditional veterinary medicine practices potentially provide effective and affordable alternatives to synthetic antibiotics in veterinary medicine.

Keywords: *Bacterial abscess, ethnoveterinary properties, Felis catus, antibacterial activities*

INTRODUCTION
Ethno medicine is an age-old practice that has evolved from collective wisdom accumulated over generations by mankind and it deals with beliefs and those practices, relating to the disease, which are the product of indigenous cultural development and not derived from the concept of modem medicines (Mahapatra *et al.,* 2019).

Ethnoveterinary medicine includes the use of medicinal plants, surgical techniques and management practices to prevent livestock diseases (Phondani *et al.,* 2010). It is defined as system of folk believes, skills, techniques and practices related to healthcare of animals that transmitted orally from generation to generation (Mc Corkle 1986). Since ethno veterinary medicine has its roots embodied in ethno botany, there is need to judiciously harvest, process, store, preserve and utilize the botanical preparation (Praveen *et al.,* 2013). These medicines differ not only from region to region but also among and within communities. Rural people especially the tribes are the rich reservoir of ethnoveterinary knowledge and practices. Ethnoveterinary medicines are cost effective and are socially compatible and easily available.

The Indian subcontinent has a rich ethnoveterinary health tradition owing to the large agriculture-based livelihoods and rich biodiversity. Medicinal plants traditionally used in the treatment of animal diseases play a crucial role in local health modalities. Specifically, phytotherapeutics often represent the primary form of therapy in rural veterinary care as allopathic modalities remain inaccessible, especially in the developing world. The utilization of medicinal plants goes way back to early people, who discovered a wealth of therapeutic agents in the Plant Kingdom and exploited their healing potential as a remedy for several animal ailments. There have been many ethnoveterinary reports from around the world regarding the use of plants in therapeutic protocol (Farooq *et al*, 2008; Graw and Eloff, 2008) and the status and prospectus of plants used in Indian ethnoveterinary medicines are well documented (Jain, 2000).

The soapnut family Sapindaceae consists of many genera, which includes plants having medicinal, ornamental, industrial and other economic values one of which is *Cardiospermum halicacabum* L. *Andrographis paniculata* is an important medicinal plant belonging to family Acanthaceae (Mishra *et al*., 2007). Traditional healers used the plant for medicinal purpose for treatment of various diseases such as gastric disorders, common colds and infectious diseases for many years (Muthu *et al.,* 2006). The plant has blood purification property used for treatment of boils, skin eruptions, scabies, leprosy, gonorrhea, chronic and seasonal fevers (Kabeeruddin, 1937). The WHO has reported that the herb *Andrographis paniculata* is widely used in Asia for cure of fever, herpes, diarrhea, inflammation, respiratory infection, throat sour, and various other infections (World Health Organisation, 2002).

The genus Tribulus, belonging to family Zygophyllaceae, comprises about 20 species of which three species, viz. *Tribulus cistoides*, *Tribulus terrestris*, and *Tribulus alatus*, are of common occurrence in India. Among them, *T. terrestris* is a well-patronized medicinal herb by Ayurvedic seers as well as by modern herbalists (Chhatre *et al*., 2014). The plant is used directly as herb or as a main component for production of a number of medicines and food supplements such as for physical rejuvenation, therapy for the conditions affecting liver, kidney, cardiovascular system and immune systems. It is also used as a folk medicine for increased muscle strength, sexual potency and in treatments of urinary infections, heart diseases and cough. It is considered invigorating stimulant, aphrodisiac, and nutritive (Saima *et al*., 2014).

Based on this information, in the current study extracts of *Cardiospermum helicacabum, Andrographis paniculata* and *Tribulus terrestris* plant extracts were used for the screening of their inhibitory potentials against the pathogens isolated from the swab samples of abscess of *Felis catus*.

MATERIALS AND METHODS

Collection and extraction of plants

The dried leaves of *Cardiospermum helicacabum, Andrographis paniculata* and *Tribulus terrestris* were collected from the local shop in Neyyattinkara, Thiruvananthapuram district in Kerala. Whole plant parts were powdered and extracted using ethyl acetate followed by methanol in sequential extraction method. The extracts were filtered using Whatman filter paper No.1 and the extracts obtained were dried and stored at 4°C for further studies.

Phytochemical screening

Phytochemical screening of the extracts was carried out to identify the phytoconstituents by following standard phytochemical assays (Sofowora, 1993).

Test for Carbohydrates

Molisch's Test

To 0.5 ml of plant extracts were treated with few drops of Molisch's reagent and few drops of concentrated sulphuric acid. Presence of purple or reddish colour indicates the presence of carbohydrates.

Test for Tannins

Ferric Chloride Test

To 0.5 ml of plant extract, 1 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

Honey Comb Test

The extracts were treated with few drops of 5% sodium bicarbonate solution. The mixture was shaken vigorously and kept for 3 minutes. Formation of honey comb like froth shows the presence of saponins.

Test for Flavonoids

Alkaline Reagent Test

0.5 ml of the extracts was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for Quinones

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones.

Test for Glycosides

To 1 ml of plant extract, 1.5 ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates the presence of glycosides.

Test for Cardiac Glycosides

To 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Test for Terpenoids

To 0.5 ml of extract, 2 ml of chloroform and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids.

Test for Phenols

To 0.5 ml of plant extract, 1 ml of 5% ferric chloride was added. Formation of blue or green colour indicates the presence of phenols.

Test for Coumarins

To 1 ml of the extract, 1 ml of 10% Sodium hydroxide was added. Formation of yellow colour indicates the presence of coumarins.

Test for Steroids and Phytosteroids

To 1 ml of the plant extract, equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid. The appearance of brown ring indicates the presence of steroids and the appearance of bluish brown ring indicates the presence of phytosteroids.

Test for Phlobatannins

To 1 ml of plant extract, few drops of 2% Hydrochloric acid were added. Appearance of red colour precipitate indicates the presence of phlobatannins.

Test for Alkaloids

To 1 ml of plant extract, 1 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent was added. Presence of green colour or white precipitate indicated the presence of alkaloids

Test for Anthraquinones

To 1 ml of plant extract, few drops of 10% ammonia solution were added. Appearance of pink colour precipitate indicates the presence of anthraquinones.

Collection of *Felis catus* abscess swab

Swab samples of abscess of *Felis catus* were collected using transportable cotton swabs from the veterinary hospital located in Thiruvananthapuram, Kerala.

Isolation of bacteria from swab samples of abscess of *Felis catus*

Collected swab was suspended in PBS and a volume of 100 µl was spread on sterile nutrient agar plates. The plates were incubated at 37°C for 24 hours. The pure bacterial cultures were isolated and refrigerated in agar slants for further studies.

Gram staining

Smear of the isolates were prepared on a clean glass slide was allowed to air dry and then heat fixed. Smear was flooded with crystal violet for one minute, it was washed with distilled water and flooded with mordant Gram's iodine, kept for one minute and again washed with distilled water. Then 1-2 drops of Gram's decolourizer was added on the smears and kept for 30 seconds and washed properly with distilled water. Then

the smear was counter stained with safranin for one minute and again washed with distilled water. The slides were then air dried and observed under light microscope and result was recorded (Coico, 2005)

Biochemical identification of isolated bacteria

Identification was performed with the Vitek 2 compact according to the manufacturer's instructions.

Antibacterial activity of plant extracts

Isolated strains were used to test the antibacterial activity of extracts of *Cardiospermum helicacabum*, *Andrographis paniculata* and *Tribulus terrestris*. The bacterial cultures were maintained in nutrient broth and kept in refrigerator at 4°C for further analysis.

Antibacterial activities of the extract were studied by the well diffusion method. Lawns of each organism were inoculated on Muller Hinton agar plates. Wells were bored by using a sterile borer and test sample with various concentrations were placed into them. Plates were incubated overnight (24-37°C). Chloramphenicol (50µg/ml) was used as the positive control and solvent was used as negative control. The antibacterial activity was determined by measuring the diameter of zone of inhibition (Kianbakht and Jahaniani, 2003).

RESULTS

The present study was focused on the evaluation of ethnoveterinary properties of *Cardiospermum helicacabum*, *Andrographis paniculata* and *Tribulus terrestris* against bacterial abscess of *Felis catus.*

Isolation of bacteria present in the swab samples of abscess of *Felis catus*

Three bacterial strains were isolated from the swab samples of abscess of *Felis catus* and named as A1, A2 and A3. (Fig 1)

Gram staining

Following that, these strains were exposed to Gram staining, which identified A1, A2, and A3 as gram-positive bacteria. (Fig 2).

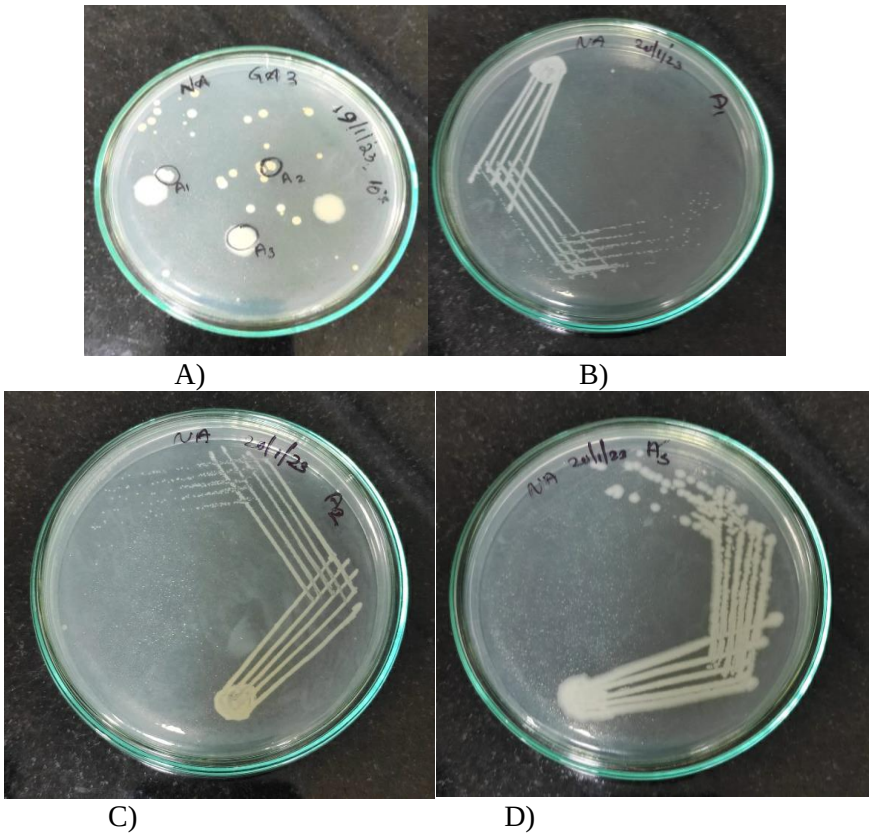
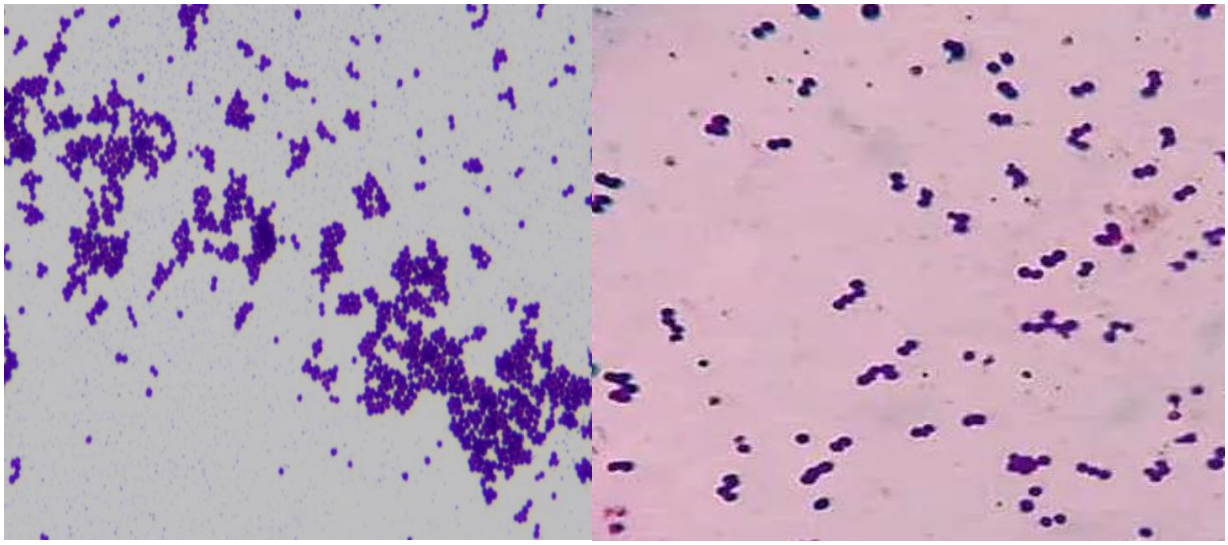
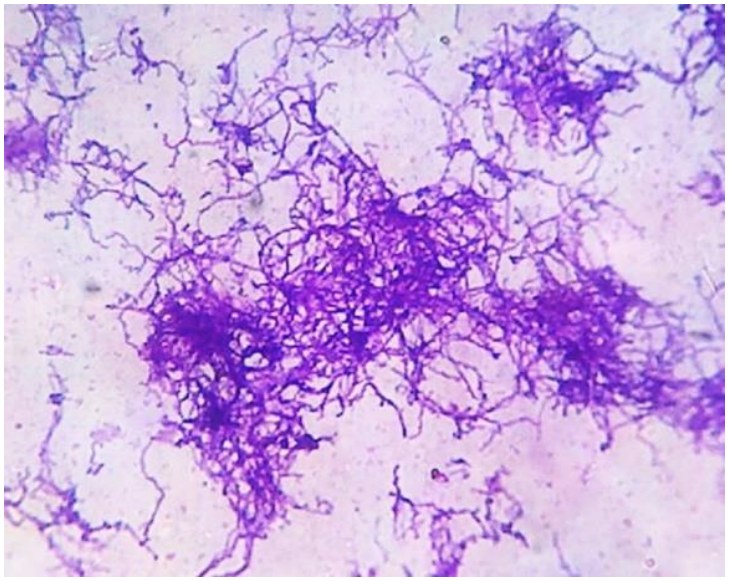


Figure 1: A) Colonies obtained by spread plate. B) Isolated colony of A1 bacteria. C) Isolated colony of A2 bacteria D) Isolated colony of A3 bacteria



A1 **A2**



A3

Figure 2: Gram staining of bacterial colonies isolated from Quadrant streak plates A1, A2 and A3.
A1 – Gram positive Cocci arranged as bunches
A2 – Gram positive Cocci arranged as individual colonies
A3 – Gram positive filamentous, thread like bacilli.

Biochemical identification of bacterial isolates

The bacterial strains isolated from abscess were identified by Vitek as ***Staphylococcus aureus*** **(A1),** ***Micrococcus varians*** **(A2),** and ***Bacillus subtilis*** **(A3).**

Qualitative phytochemical analysis of plant extracts

The phytochemical characteristics of ethyl acetate and methanol extracts of *C. helicacabum*, *A. paniculata* and *T. Terrestris* are summarized in Table 1. The results revealed the presence of medically active compounds in the three plants studied. Carbohydrates, phenols and alkaloids were present in all the plants.

Antibacterial activity of Plant extracts

Methanolic extract of *Cardiospermum halicacabum* and Ethyl acetate extract of *Andrographis paniculata* showcased highest antibacterial activity. A1 and A2 strains showed high resistance against both plant extracts,

A3 strain showcased sensitivity to both the plant extracts. (Table 2) When compared, *Andrographis paniculata* showcased more potential ethnoveterinary activity.

Table 1: Phytochemical analysis of *C. helicacabum, A. paniculata* and *T. Terrestris*

Phytochemical Tests	**Phytochemical Result**					
	C. helicacabum		***A. paniculata***		***T. Terrestris***	
	Ethyl acetate	**Methanol**	**Ethyl acetate**	**Methanol**	**Ethyl acetate**	**Methanol**
Carbohydrates	+	+	+	+	+	+
Tannins	+	+	+	+	-	-
Saponins	-	-	+	-	-	-
Flavonoids	-	+	+	-	+	+
Alkaloids	+	+	+	+	+	+
Quinones	+	+	-	+	+	-
Glycosides	+	+	-	+	+	-
Cardiac glycosides	+	-	-	+	-	+
Terpenoids	-	-	-	-	-	-
Phenols	+	+	+	+	+	+
Coumarins	-	-	-	-	+	-
Steroids	-	-	-	-	-	-
Phytosteroids	+	-	+	+	+	+
Phlobatannins	-	+	-	+	+	+
Anthraquinones	-	-	-	-	-	-

+ indicates presence and – indicates absence of phytochemical

Table 2: Antibacterial activity of ethyl acetate and methanol extracts of *Cardiospermum halicacabum, Andrographis paniculata,* and *Tribulus terrestris*.

Bacterial isolates	**Solvent used**	**Concentration of extracts (µg/ml)**	**Zone of inhibition in control (cm)**	**Zone of Inhibition with Plant extracts (cm)**		
				Cardiospermum helicacabum	*Andrographis paniculata*	*Tribulus terrestris*
A1	Ethyl acetate	50	1.2	1.1	1.2	0.8
		100	1.7	1	1.2	1.3
	Methanol	50	1.9	1.1	1.1	1.3
		100	1.6	0.9	0.8	1
A2	Ethyl acetate	50	1.3	1	1	1
		100	1.6	1.2	1.4	1.3
	Methanol	50	1.6	1.1	1.3	1.1
		100	1.4	1.1	1.1	1
A3	Ethyl acetate	50	1.3	1.1	1	1.2
		100	1.6	1.3	1.3	1.2
	Methanol	50	1.2	1.3	1	1.1
		100	1.4	1.4	1.2	0.9

DISCUSSION

Knowledge of plant chemistry is very much essential for the development of useful plant products. In fact, there are thousands of plants that have the potential to yield drugs of great use to man but are still unexplored. The present study is focused on the evaluation of ethnoveterinary properties of *Cardiospermum helicacabum*, *Andrographis paniculata* and *Tribulus terrestris* against bacterial abscess of *Felis catus.* In this study, methanol extract of *Andrographis paniculata* showed a higher antibacterial activity against all bacterial isolates. This may be due to the presence of phytochemical constituents present in it. Majority of phytochemical compounds namely terpenoids, alkaloids, saponin, steroids and anthraquinones are mainly involved in antimicrobial activity. Meanwhile, phenolic and flavonoids compounds are found to have antioxidant property.

Although the specific compounds that attribute to the antibacterial activity have not been determined in this study, numerous studies have reported on the antimicrobial activity of alkaloids from several medicinal plants such as *Jatropha curcas*, *Calotropis procera*, *Carica papaya*, *Magnifera indica* and *Psidium guajava* (Doughari, 2009; Garba and Okeniyi, 2012). Flavonoids are hydroxylated phenolic compounds that have antimicrobial activity against a wide range of microorganisms (Yadav and Agarwala, 2011). In addition, they are antioxidants that act as free radical scavenger (Carocho and Ferreira, 2013). The phytochemicals content in the plant are constantly found to be fluctuated with the genetic heterogeneity of a plant species. The genetic heterogeneity might be caused by the variation in soil condition, seasonal cycle, climate, weather, age of plant, sun and shade fluctuations (Joshua and Takudzwa, 2013).

In addition, this finding is contradictory to the finding of Sule, *et al.*, (2011) who reported that the methanol extract of *A. paniculata* showed a high antibacterial activity against *S. aureus* and *S. pyogenes*. Premanath and Devi (2011) had also reported that the hexane, chloroform and methanol extracts of *A. paniculata* showed antibacterial activity against *E. coli* by agar disc diffusion method.

Similarly, Deepan *et al*., (2012) reported that the methanolic extract *Cardiospermum halicacabum* L are effective to Gram-negative *Klebsiella pneumoniae*. The aqueous and alcoholic extracts showed this activity but the former one has shown better activity and the zone of inhibition increased with increase of concentration (Deepan *et al*., 2012).

Suresh *et al*., (2012) performed the analysis of *in-vitro* antibacterial activity of *Cardiospermum halicacabum* L., by agar disc diffusion method. Among this *Staphylococcus aureus* and *Bacillus subtilis* gave the high zone of inhibition

In the present study, bacterial strains A1 and A2 showed resistance in both methanolic extracts of *Cardiospermum halicacabum, Andrographis paniculata* and *Tribulus terrestris.* Further, isolating the bioactive compounds and determining the mode of action through in vitro and *In silico* studies are essential. Moreover, future studies are essential to elucidate the structural interactions of the active compounds and to evaluate its pharmacological properties.

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