

Phytochemical Screening and Antimicrobial Activity of Medicinal Plants in Hadoti Region of Rajasthan

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Received: 2.10.2020 | Revised: 4.11.2020 | Accepted: 10.11.2020

ABSTRACT

Phytochemicals are bioactive compounds obtained from the plants, widely used and applied in the traditional herbal medicines. These herbal medicines are the main natural source for the local people to cure the various diseases including the major diseases such as diabetes mellitus, cardiovascular disease, inflammation, neurologic disorders, HIV and cancer. The results of study revealed the natural curative agents with an extra-ordinary safety profile due to the presence of alkaloids, flavonoid and saponins. The medicinal plants are still considered as one of the peremptory parts of modern medicine. Therefore, the driven force of this research was to analyse the phytochemical activity of selected plant species against bacterial pathogens Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia. It was concluded that the plants screened in this research were rich in phytochemicals with significant pharmacological and medicinal applications.

Keywords: *Phytochemical, Anti-microbial, Antioxidant, Saponin, Alkaloid, Flavonoid.*

INTRODUCTION

Phytochemicals generally originated from the plant sources are the bioactive compounds which are also known as secondary metabolites. Primary metabolites are important for the plant's regular metabolic activities such as growth and development. Secondary metabolites produced by plants may have little need for them. These are synthesized in almost all parts of the plant like bark, leaves, stem,

root, flower, fruits, seeds etc. From ancient era to past several years, phytochemicals have been used world-wide as the traditional herbal medicine (Seinivasen et al., 2007). Because of these pharmaceutical industries as well as researchers put a greater emphasis on the phytochemical studies. Also, these phytochemicals present in the different plant parts were used up by the local people for healing of certain disorders from past.

Cite this article: Tiwari, R., Sharma, A., Mehra, N., Kumar, V., Nimmy, M. S., & Meena, R.K. (2020). Phytochemical Screening and Antimicrobial Activity of Medicinal Plants in Hadoti Region of Rajasthan, *Ind. J. Pure App. Biosci.* 8(6), 90-99. doi: <http://dx.doi.org/10.18782/2582-2845.8387>

Secondary metabolites are economically important and used in the production of drugs derived from plants which are simple synthetics modification, and naturally obtained substances. Natural products are a source of new chemical diversity and are choice of the world. They are primarily required materials for health care system in all most all part of the world. There is a growing research interest in plants as a therapeutic agent.

The therapeutic properties of plant products can be traced back to over five thousand years ago as there is evidence of its use in curing diseases, rejuvenation and for revitalizing by systems in Indian, Egyptian, Chinese, Greek and Roman Civilizations (Mahesh & Salish, 2008). India has about 4-5 million plant species and among them estimated only 250,000-500,000 plant species, have been investigated for pharmacological activity. Many medicinal plants as a source of new therapeutics are yet to be explored. The importance of the medicinal plant in drug development is well known science to mankind; humans were used to curing different diseases by using medicinal plants from the beginning of human development (Fransworth et al., 2008). Treatment from wild plants species and their resistance towards several microbial cells has always guided researchers to search for novel medication strategies to provide healthy lifestyle for humans in addition to some medicinal plants are still obscured within the plant which need to be scientifically evaluated (Achlerberg et al., 2013). Characterization of several active phyto compounds and their validation from these green natural factories has given birth to some high activity profile drugs (Mandal et al., 2007). Several evidences indicate that secondary plant metabolites play impassable and crucial roles in human health and are nutritionally important. It is believed and reported by researchers that crude extract from medicinal plants are more biologically active than isolated compounds; due to their

synergistic effects (Jana & Shekhawat, 2010). Phytochemical screening of plants was revealed the presence of numerous chemicals including alkaloid, tannins, flavonoids, steroids, glycosides and saponins etc. Plant products have been part of phyto-medicines since time ancient to till today. These can be derived from different plant part like-bark, leaves, flowers, roots fruit, seeds etc.; phytochemical screening of various plants were reported by many workers (Mojab et al., 2003).

The basic parameters influencing the quality of an extract (Ncube et al., 2008) are the plant part which is used as starting material, solvent used for extraction and extraction procedure. The effect of extracted plant phytochemicals depends on different parameters: the nature of the plant material, its origin, degree of processing, moisture content and particle size. The variations in different extraction methods can affect quality of secondary metabolite. Composition of an extract depends on different parameters like type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and polarity. The bioactive compound mostly found in plants such as phenolics and polyphones (Shi et al., 2005), are known as secondary metabolites. Important subclasses in this group compounds which had been found to have antimicrobial activity include phenols, phenolic acid, quinones, flavones, tannins and coumarins (Orphanides et al., 2013). This group is derived from the condensation of acetate units (terpenoids) those produced by the modification of aromatic amino acid (phenyl propanoids and coumaris). Flavonoids and tannins are plant-based antioxidant cannot be synthesized by animals. This was evident traditionally, that phenolics were used for protection of inflamed surface of the mouth and treatment of the wounds, haemorrhoids and diarrhoea (Ogunleye and Ibitoye, 2003).

The antimicrobial activity of different phenolics had been reported earlier. They were extracted from plant resources and their modes of action were explained by many workers

1. The position and number of hydroxyl groups was related to the level of toxicity in case of some phenols e.g. carvacrol.
2. Mechanisms of action: phenolic acids, flavonoids and tannins involve enzyme inhibition, enzyme inactivation, formation of complexes with cell walls and metal.
3. Disruption of cell homeostasis leading to growth inhibition and cell death.

Plant based natural constituents can be derived from any plant part like bark, leaves, flowers, roots, fruits, seeds etc. i.e. any part of the plant may contain active components. The systematic screening of plant species with the aim of discovering new bioactive compounds and investigating their activity was a routine activity in many laboratories from last decades. Plants were collected either randomly or by following leads supplied by local healers in geographical areas where the plant were found (Lims et al., 2006). Many authors had been reported about plant extract preparation from the fresh plant tissues. The ethno-medicinal uses of fresh plant materials among the traditional practitioners were popular. Plants were used in the drug form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissue, plants were usually air dried to a constant weight before extraction. Some researchers dry the plants in the oven at about 40°C for 72h. In most of the reported works, underground parts (roots, tuber, rhizome, bulb, etc) of a plant were used extensively compared with other above ground parts in search for bioactive compounds processing antimicrobial properties (Ncube, 2008 & Dask, 2010).

Structural characterization

The purified compound needs to be structurally determined. This involves accumulating data from a wide range of spectroscopy techniques, such as UV-visible spectroscopy, infra-red and nuclear magnetic methods.

Antioxidant property of bioactive compound

The resonance which gives some although almost all parts of the electromagnetic spectrum was used for studying matter in organic chemistry, but natural products are concerned with energy absorption from there or four regions—Ultraviolet, visible, infrared, radio frequency, and electron beam. Nature of the compound can be determined making use of UV-visible spectroscopy. Antioxidant compounds in natural products play an important role in health protecting sector. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases (Fitriansysh et al., 2018). Most of the antioxidant compounds were derived from plant sources and belong to various classes of compounds with a wide variety of physical, biochemical and clinical properties. The main characteristic of an antioxidant was its ability to trap free radicals, these highly reactive free radicals and oxygen species are present in any biological system from a wide variety of sources. Antioxidant compounds like phenolic acids, polyphenols and flavonids scavenged free radicals such as peroxide or hydro peroxide, thus inhibit the oxidative mechanisms in nucleic acids proteins, lipids or DNA and can initiate.

Toxicity of bioactive Compound

These compounds play a protective role by eliminating potential mutants. Other than that, it can also be used as herbicide (Sumthong, 2007), cosmetics (Dharmanda, 2003), as pain killer as medicines in hypertension (Edema & Alaga, 2012). The plant and products were safer than the synthetic one's fact is that they are neither safe nor completely poisonous in low amounts. Whereas high dose and prolonged use may be harmful (Jowell, 1999). The toxicity in using plant products may be due to various reasons:

1. Inappropriate plant identification
2. Use of plants that interfere with biological systems such as plants containing coumarone derivative, allergic plant, plants containing photosensitive compound.

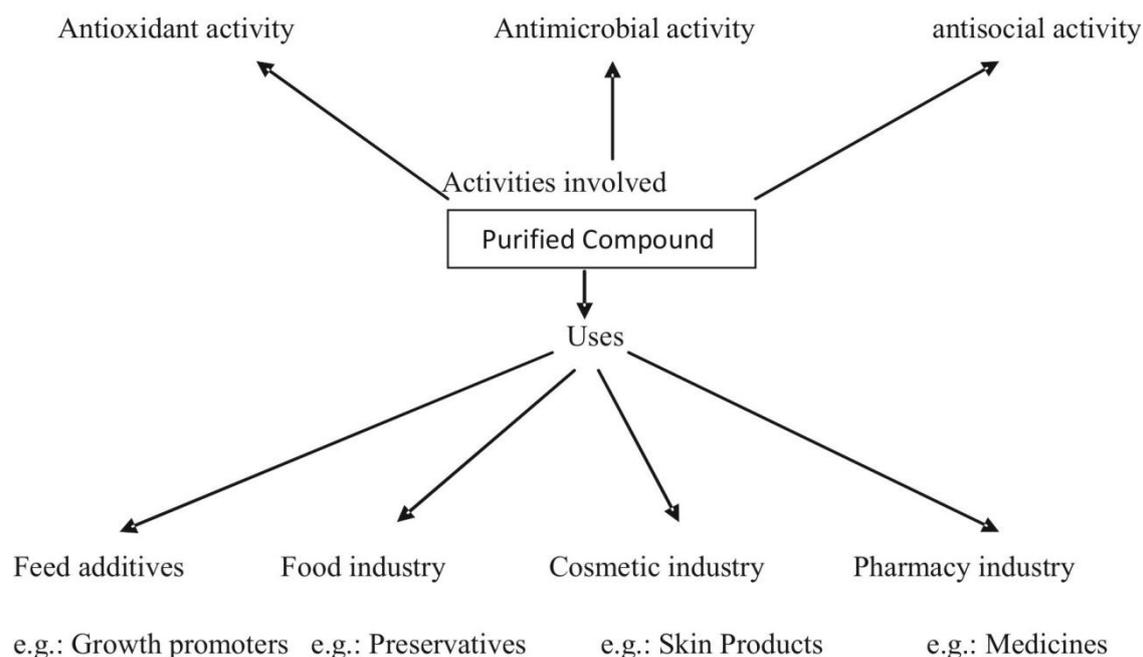


Fig. 1: The various application of a purified bioactive molecule from plant (Vasan, 2009)

MATERIALS AND METHODS

Plant Material

The following medicinal plants were selected for the study from the local area based on their basic information available. *Eupatorium glandulosum*, *Abutilon indicum*, *Datura stramonium* and *Lantana camara*. Fresh samples of plants were collected, washed and air dried. The dried leaves were powdered and stored in air tight bottles separately for further studies

Preparation of Plant Extract

Aqueous Extraction

Samples of 10gm were immersed in 100 ml of distilled water, mixed and allowed to soak for 24 hours. Then the mixer was filtered through Whatmann No.4 filter paper to get pure extract.

Methanol Extraction

Air dried powder of 10gm was placed in a conical flask containing 100 ml of organic solvent (Methanol) plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 hours. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and then solvent was evaporated to make the upto final volume one-fourth of its original volume.

Ethanol Extraction

Ten grams of sample was soaked in 100 ml of 95% ethanol and kept in room temperature for 24 hours. Then the extract solution was filtered through a Whatmann No.4 filter paper. Then the solvent was removed using a rotary vacuum evaporator until it reaches one-fourth of its volume. All the above extracts were stored at 4°C in air tight bottles for further studies.

Microorganisms

The investigated microorganisms consisted of two Gram-positive bacteria *Staphylococcus aureus* ATCC25923, *Klebsiella spp* ATCC13883; one Gram negative bacterium: *Escherichia coli* ATCC25922. Microorganisms were obtained from the Maharao Bhim singh Hospital, Kota (MBS), microorganisms were maintained at 4°C on nutrient agar slants.

Antimicrobial susceptibility test

The antimicrobial assay was performed by the agar disc diffusion method (Bauer et al., 2017). The 20 ml of sterilized Muller Hinton Agar was poured into sterile petri plates, after solidification, 100 µl of fresh bacterial culture were swabbed on the respective plates. Each of disc which was approximately 5 mm in diameter cut from Whatmann filter paper. The

sterile discs were kept over the agar plates using sterile forceps at various concentrations (2, 4, 6, 8, and 10 µl). The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each disc was measured (mm) and data was recorded (Nair et al., 2005).

RESULTS AND DISCUSSION

Antimicrobial potential of plants was compared according to their zone of inhibition against the several pathogenic organisms. The ethno-botanical information of four traditionally used Indian plant species is given in table 1 which were selected for antibacterial activity in this research.

Table 1: Ethnobotanical information of some traditionally used plant species

S. No.	Plant species	Family	Common name
1.	<i>Eupatorium glandulosum</i>	Asteraceae	Black bamboo
2.	<i>Abutilon indicum</i>	Malvaceae	Kanghi
3.	<i>Lantana camara</i>	Verbenaceae	Lantana
4.	<i>Datura stramonium</i>	Solanaceae	Datura

The result obtained for the antimicrobial test performed on different extract of medicinal plants and zone of inhibition of the individual plant extract with three bacterial pathogens

(*Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*) were identified as given in table 2, table 3 and table 4.

Table 2: Zone of inhibition of individual plant extracts with bacterial pathogen *E. coli*.

Bacteria	Plants	1% Extract solutions Concentration (µl) and zone in (cm)								
		Alkaloids			Flavonoids			Saponins		
		6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)
<i>Escherichia coli</i>	<i>Eupatorium glandulosum</i>	1.6	1.8	1.9	1.3	1.5	1.7	0.9	1.1	1.3
	<i>Abutilon indicum</i>	1.4	1.6	1.7	1.4	1.5	1.7	0.7	0.9	1.1
	<i>Lantana camara</i>	1.9	1.9	2.2	1.6	1.9	2.2	1.1	1.3	1.3
	<i>Datura stramonium</i>	1.4	1.8	2.2	1.9	2.0	1.3	0.8	1.0	1.3

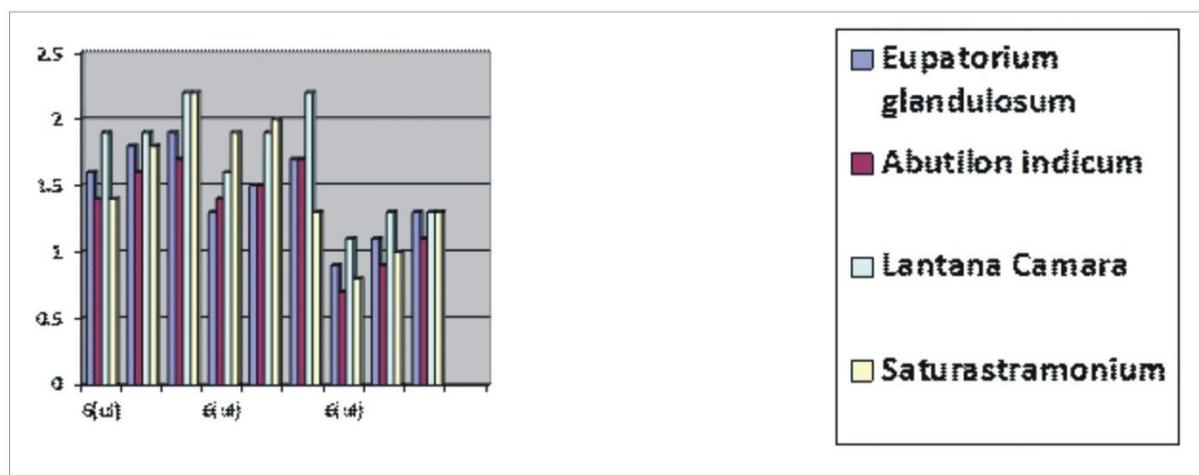


Fig. 2: The graphical representation of alkaloids, flavonoids and Saponins antibacterial test for *Escherichia coli*

Alkaloids antibacterial susceptibility test results for *Escherichia coli*, conclude that the *Datura stramonium* and *Lantana camara* show the significance zone of inhibition that is the highest zone inhibition 2.2 cm in the concentration of 10 µl. *Eupatorium glandulosum* 1.9 cm was noted in 10 µl (table 2 and fig. 2).

Flavonoids antibacterial susceptibility test result for *Escherichia coli* with four medicinally important plants showed that the highest zone of inhibition 1.6 cm was noted in the concentration of 6 µl, 1.9 cm in 8 µl and 2.2 cm in the concentration of 10 µl for *Lantana camara*. The next highest zone of inhibition observed in the *Datura stramonium*, 1.6 cm in 6 µl, 1.9 cm in 8 µl and 2.2 cm in the concentration of 10 µl; and in *Abutilon indicum*, 1.4 cm in 6µl, 1.5 cm in 8 µl and 1.7 cm was noted in the 10 µl. *Eupatorium glandulosum* showed the less zone of inhibition against *E.coli*, 1.3 cm in 6 µl, 1.5 cm

in 8 µl and 1.7 cm was noted in the concentration of 10 µl. From the above observation given in table 2 and fig. 2, *Lantana camara* showed the significance zone of inhibition against the *Escherichia coli*.

Saponins antibacterial susceptibility test result for *Escherichia coli*, showed that the highest zone of inhibition 1.8 cm was noted in the concentration of 6 µl, 1.0 cm in 8 µl and 1.3 cm in the concentration of 10 µl for *Datura stramonium*. The second highest zone of inhibition was showed by *Abutilon indicum*, 0.7 cm in 6 µl, 1.9 cm in 8 µl and 1.1 cm was noted in the concentration of 10 µl. Third highest zone of inhibition was showed by *Eupatorium glandulosum*, 0.9 cm in 6 µl, 1.1 cm in 8 µl and 1.3 cm was noted in the concentration of 10 µl. *Lantana camara* showed the less zone of inhibition against *E. coli*. From the above observation given in table 2 and fig. 2, *Datura stramonium* showed the significance zone of inhibition against *E. coli*.

Table 3: Zone of inhibition of individual plant extracts with bacterial pathogen *Staphylococcus aureus*

Bacteria	Plants	1% Extract solutions Concentration (µl) and zone in (cm)								
		Alkaloids			Flavonoids			Saponins		
		6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)
<i>Staphylococcus aureus</i>	<i>Eupatorium glandulosum</i>	2.1	2.4	2.7	1.5	1.7	1.9	1.7	1.9	2.1
	<i>Abutilon indicum</i>	2.2	2.3	2.5	1.7	1.9	2.1	1.1	1.2	1.3
	<i>Lantana camara</i>	2.4	2.6	2.8	2.2	2.5	2.7	1.5	1.7	1.9
	<i>Datura stramonium</i>	2.7	2.8	1.9	1.9	2.1	2.2	1.5	1.7	1.9

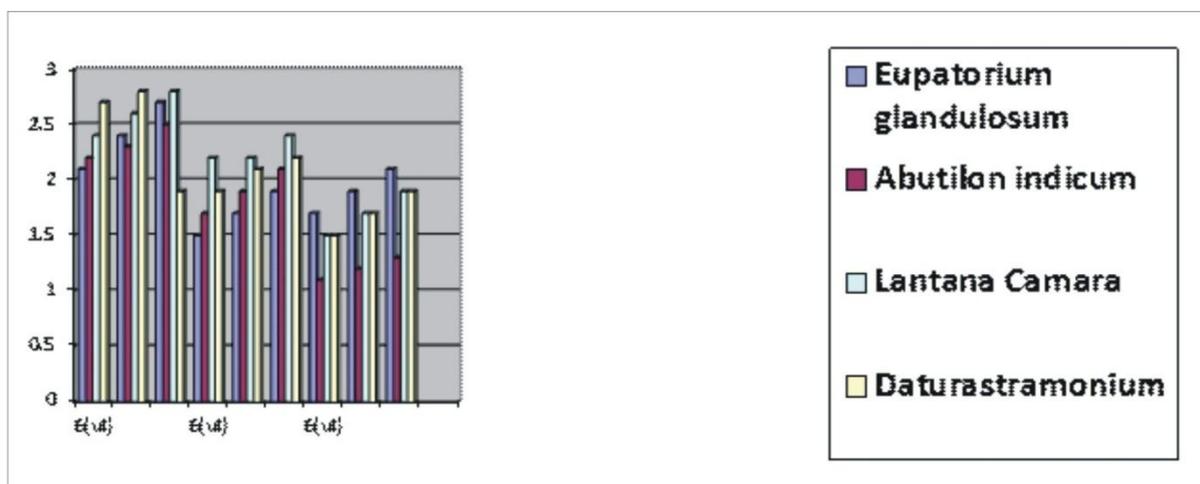


Fig. 3: Represent the Alkaloids, Flavonoids, Saponins antibacterial Test for *Staphylococcus aureus*

Alkaloids antibacterial susceptibility test result for *Staphylococcus aureus* given in table 3 and fig. 3, showed that the significance zone of inhibition 2.8 cm was noted in the concentration of 10 µl and 2.6 cm noted in the 8 µl and 2.4 for 6 µl for *Lantana camara*. *Datura stramonium* showed the second highest zone of inhibition 1.9 cm in the concentration of 10 µl. For *Abutilon indicum*, 2.5 cm in the concentration of 10 µl was observed. *Eupatorium glandulosum* showed 2.7 cm in concentration of 10 µl. From the above observation *Lantana camara* showed the significance zone of inhibition.

Flavonoids antibacterial susceptibility test result for *Staphylococcus aureus* as showed in table 3 and fig. 3; the highest zone of inhibition 1.9 cm was noted in the concentration of 6 µl, 2.1 cm in 8 µl and 2.2 cm in the concentration of 10 µl for *Datura stramonium*. The next highest zone of inhibition observed in the *Lantana camara*, 2.2 cm in 6 µl , 2.5 cm in 8 µl and 2.7 cm was

noted in the concentration of 10 µl. *Abutilon indicum* showed the moderate zone of inhibition 1.7 cm in 6 µl , 1.9 cm in 8 µl and 2.1 cm was noted in the concentration of 10 µl. *Eupatorium glandulosum* showed the less zone of inhibition against *Staphylococcus aureus*. The observation given in table 3 and fig. 3, *Datura stramonium* showed the significance zone of inhibition again *Staphylococcus aureus*.

Saponins antibacterial susceptibility test result for *Staphylococcus aureus* showed the highest zone of inhibition 1.1 cm was noted in the concentration of 6 µl, 1.2 cm in 8 µl and 1.3 cm in the concentration of 10 µl for *Abutilon indicum*. The second highest zone of inhibition was showed by *Datura stramonium* and *Lantana camara* the less zone of inhibition was noted. From the above observation given in table 3 and fig. 3, *Abutilon indicum* showed the significance zone of inhibition against *Staphylococcus aureus*.

Table 4: Represent the Alkaloids, Flavonoids, Saponins antibacterial test for *Klebsiella pneumonia*

Bacteria	Plants	1% Extract solutions Concentration (µl) and zone in (cm)								
		Alkaloids			Flavonoids			Saponins		
		6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)	6(µl)	8(µl)	10(µl)
<i>Klebsiella pneumonia</i>	<i>Eupatorium glandulosum</i>	1.9	2.1	2.3	1.5	1.7	1.8	1.2	1.3	1.5
	<i>Abutilon indicum</i>	1.5	1.7	1.9	1.7	1.9	2.1	1.1	1.2	1.3
	<i>Lantana camara</i>	2.4	2.3	2.5	2.5	2.5	2.7	1.7	1.7	1.9
	<i>Datura stramonium</i>	2.5	2.5	2.7	1.3	2.1	2.6	1.7	1.4	1.7

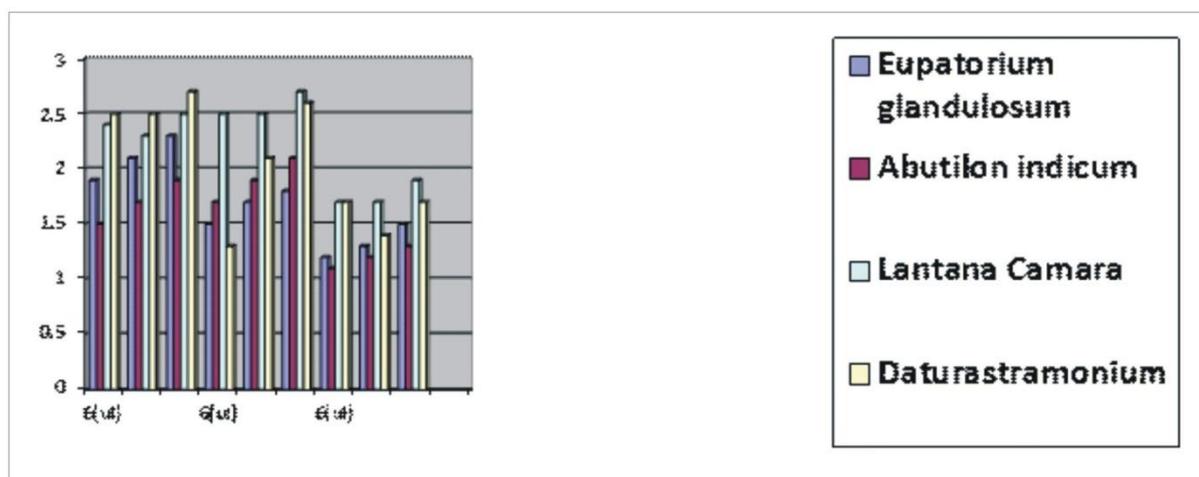


Fig. 4: The graphical representation of alkaloids, flavonoids and Saponins antibacterial test for *Klebsiella pneumonia*

Alkaloids antibacterial susceptibility test result (table 4 and fig. 4), for *Klebsiella pneumonia* showed the highest zone of inhibition 2.5 cm was noted in the concentration of 10 µl for *Lantana camara*. For *Datura stramonium*, 2.7 cm was noted in the concentration of 10 µl and for *Abutilon indicum*, 1.9 cm concentration of 10 µl. From the above observation *Lantana camara* showed the significance zone of inhibition against the *Klebsiella pneumonia*.

Flavonoids antibacterial susceptibility test result (table 4 and fig. 4), for *Klebsiella pneumonia* showed the highest zone of inhibition 1.3 cm was noted in the concentration of 6 µl, 2.1 cm in 8 µl and 2.6 cm in the concentration of 10 µl for *Datura stramonium*. The next highest zone of inhibition was observed in the *Lantana camara*, 2.5 cm in 6 µl, 2.5 cm in 8 µl and 2.7 cm was noted in the concentration of 10 µl. *Abutilon indicum* showed the moderate zone of inhibition. From the above observation *Datura stramonium* showed the significance zone of inhibition against *Klebsiella pneumonia*.

Saponins antibacterial susceptibility test result for *Klebsiella pneumonia* showed the highest zone of inhibition 1.7 cm was noted in the concentration of 6 µl, 1.4 cm in 8 µl and 1.9 cm in the concentration of 10 µl for *Datura stramonium*. The second highest zone of inhibition was showed by the *Lantana camara*, 1.7 cm in 6 µl, 1.7 cm in 8 µl and 1.9 cm was noted in the concentration of 10 µl. The above observations given in table 4 and fig. 4, *Datura stramonium* showed the significant zone of inhibition against *Klebsiella pneumonia*. From the above observation it was noted that the alkaloids and flavonoids fractions showing the significant zone inhibition compared to the saponins fraction.

Many antibiotics are used now a days to control the diseases. Increased awareness of the environmental problems associated with these antibiotics have led the search for non-conventional chemicals of biological origin. Bactericides of plant origin can be one approach for disease (Mezaine et al., 2005). Screening is important not only to uncover

therapeutic efficacy of the medicinal plants, but also to understand their historical utilization by traditional healers and herbalists. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures boast large contributions to health and well-being. The utilization of plant extracts with recognized antimicrobial properties can be of great significance for therapeutic treatment. The present study was aimed to validate plants as source of potential chemotherapeutic agents and antimicrobial activity; in past also, researchers tried to validate plant as a new antimicrobial agent (Cledson et al., 2007). The search of novel bioactive compounds including antimicrobial agent continues till 2020. This is largely so because some pathogens have developed resistance to certain currently used drugs and some disease have yet to be treated chemotherapeutically (Chin et al., 2006). Medicinal plants are stipulated for the scientific vision, to establish a balanced relationship between chemical, biological and therapeutically activities of folklore medicine.

CONCLUSION

In this study identified the antimicrobial properties of medicinal plants (*Eupatorium glandulosum*, *Abutilon indicum*, *Datura stramonium* and *Lantana camara*.) on account of the presence of following phytochemicals namely alkaloids, flavonoids and saponins. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism. Results obtained from the current work, indicate that, the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics; alkaloids and flavonoids were biologically active, against *E. coli*, *S. aureus* and *Klebsiella pneumonia*. The antimicrobial activity of methanolic extract of *Datura stramonium* and *Lantana camara* showed the significant antimicrobial activity towards selected pathogens *E. coli*, *S. aureus* and *K. pneumonia*. These plant extracts are used as a potential and alternative approach for human

health in future with reduced side effects. The cost and benefits of these new herbal solutions will be a new area of exploration in field of biotechnology and microbiology.

Acknowledgement

Author thanks to Maharao Bhimsingh Hospital (MBS), Kota and Department of Botany, University of Rajasthan, Jaipur to provide continuous support and Laboratory facilities during this research.

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