Journal of Bioresource Management

Volume 4 | Issue 4 Article 1

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Recommended Citation

Minhas, S. A., Khan, F. M., Abbas, F., & Faiz, A. H. (2017). Phytochemical Screening and Determination of Antibacterial, Anti-Tumorigenic and DNA Protection Ability of Root Extracts of Saussurea Lappa, *Journal of Bioresource Management, 4* (4).
DOI: https://doi.org/10.35691/JBM.7102.0077
ISSN: 2309-3854 online
(Received: Sep 15, 2019; Accepted: Sep 17, 2019; Published: Sep 20, 2017)

Phytochemical Screening and Determination of Antibacterial, Anti-Tumorigenic and DNA Protection Ability of Root Extracts of Saussurea Lappa

PHYTOCHEMCIAL SCREENING AND DETERMINATION OF ANTIBACTERIAL, ANTI-TUMORIGENIC AND DNA PROTECTION ABILITY OF ROOT EXTRACTS OF *SAUSSUREA LAPPA*

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ABSTRACT

Saussurea lappa, a traditionally well-known plant has been used in different indigenous systems of medicine because of medicinal values. It is widely used in the treatment of asthma, ulcer, stomach problems and inflammatory diseases. In current study the phytochemical screening of *S. lappa* showed the presence of sesquiterpenes, anthraquinones, lignans, alkaloids, flavonoids, glycosides and steroids. Antimicrobial potential of subject plant was also investigated against three bacterial strains and maximum antibacterial activity was observed by CEE against *S. aureus*, CAE against *P. aeruginosa*, while CME, CEE, EAE and PEE showed almost same significant activity against *E. coli*. The CME (1000 ppm) was found to have maximum anti-tumor activity with 75% tumor inhibition potential. Significant DNA protection (of plasmid pBR322) activity was observed in 100 ppm CME and EAE. Therefore, *S. lappa* can be considered as promising candidate for isolation of bioactive compounds having antibacterial, antitumor and DNA protective potential.

Keywords: antibacterial, anti-tumor, DNA protection, *Saussurea lappa*, phytochemicals

Abbreviations: CME: Crude methanolic extract, CEE: Crude ethanolic extract, CAE: Crude aqueous extract, EAE: Ethyl acetate extract, PEE: Petroleum ether extract

INTRODUCTION

Plants have been traditionally used for cancer treatment since ancient times. Isolation of potent anticancer agents including vincristine and vinblastine from *Catharanthus roseus* attracted the attention of researches for investigating anti-tumor potential of folklore plants (Cragg and Newman, 2005). Plants are also being widely used to cure infectious diseases as well. Plants are reservoir of secondary metabolites such as terpenoids, tannins, flavonoids and alkaloids which have been found to possess antimicrobial property (Cowan, 1999).

Saussurea lappa is the member of the family Asteraceae having 300 species (Pandey et al., 2007). Among them, *S. lappa* is a representative tall and robust perennial herb. This specie grows on moist open slopes between the altitudes of 2600-3600 m (Zahara et al., 2014). Highest diversity is in the alpine habitats of the Himalaya and Central Asia. In Pakistan, it is mainly found in Azad Kashmir, Kaghan and Northern areas (Aswal and Mehrotra, 1994).

The medicinal value of *S. lappa* lies in some biochemical active substances that produce different physiological actions on human body. These biochemically active constituent are alkaloids, sesquiterpenes, tannins, flavonoids, phlobatanins, phenolic compounds, carbohydrates and glycosides (Chen et al., 1995; Simha et al., 2008; Sutar et al., 2011). *S. lappa* is traditionally used as aromatic, thermogenic, deodorant, digestive, carminative, anodyne,

aphrodisiac and stomachic. Stem and flowers of this plant have also been used for a long time in traditional Chinese medicines for the treatment of stomachache, dysmenorrheal, altitude sickness rheumatoid arthritis and cough with cold (Pandey et al., 2007; Sudhir et al., 2010; Madhuri et al., 2012; Lin et al., 2015).

Phytoconstituents are attributable for various pharmacological actions including anti-inflammatory, antiulcer, immunostimulant, antimicrobial, neurotoxic and cardiotonic activity (Kulkarni, 2001; Tian and Hua, 2005; Pandey et al., 2007; Parekh and Chanda, 2007). *S. lappa* has been found to inhibit the mRNA expression of NOS (nitric oxide species) by lipopolysaccharides which stimulates the macrophages resulting in decreased production of nitric oxide (Sun et al., 2003). In the view of these aforementioned aspects current research was conducted to investigate the presence of phytoconstituents and antibacterial, anti-tumor and DNA protection potential of *Saussurea lappa*; a folklore plant.

MATERIALS AND METHODS

Sample Collection

The roots of the plant were collected from Azad Jammu and Kashmir (AJK), Pakistan and were air dried under shade at room temperature. The roots were crumbled and kept in an air tight container. Morphology of plant has been shown in Figure 1.

Preparation of Crude Extracts

Crude extracts of methanol, ethanol and water were prepared. For this purpose 1.5 g of each sample was accurately weighed and dissolved in 50 mL of 100% methanol and ethanol separately. Samples were then kept in orbital shaker at 150 rpm for a week. It was followed by filtration and filtrates were kept at 40 °C allowing the solvent to evaporate leaving behind the dried crude extracts. The crude aqueous extract was also prepared by following the aforementioned protocol.

a b

Figure 1: *Saussurea lappa*; (a) Dried roots, (b) Fresh herb.

Preparation of Soxhlet Extract

Two soxhlet extracts i.e., ethyl acetate and petroleum ether extracts were prepared. Extraction was done by using 200 mL of petroleum ether and ethyl acetate in soxhlet extractor. The temperature was kept less <77 °C and <90-100 °C respectively for both extracts. Samples were then collected in the collection flasks and stored at room temperature.

Phytochemical Analysis

Phytochemical assays were performed for tannins, saponins, anthraquinones, amino acids, lignans, alkaloids, steroids, sesquiterpenes, glycosides and flavonoids following the published protocol (Akhtar and Farah, 1987).

Biological Activities

i. Antibacterial Activity

Antibacterial activity of test samples against two gram negative (*E. coli* and *P. aeruginosa*) and one gram positive (*S. aureus*) strain was determined by following the previously used disc diffusion method with slight modifications (Nimri et al., 1999; Shafique and Qureshi,

2015). Test samples (CME, CEE, CAE, EAE and PEE) of 20 µg/mL and 40 µg/mL concentrations were used against each strain. Cefixime was employed as positive control while DMSO was employed as negative control. Zones of inhibition were measured after 24 hours.

ii. Antitumor Assay

Anti-tumor assay was performed by following the previously illustrated protocol (Coker et al., 2003). *Agrobacterium tumefaciens* culture was used for tumor induction in potato discs. Plant extracts of 10, 100 and 1000 ppm were prepared in DMSO. Camptothecin (Sigma Chemical Co., St. Louis, MO) and DMSO were used as positive and negative controls, respectively. Culture plates were incubated at 28°C for 21 days for tumor induction. Lugol's Reagent was used for staining procedure after incubation period. Potato discs were examined using dissecting microscope and inhibition percentage was calculated the formula given below;

$$\% \text{ Inhibition} = (1-Ns/Nc) \times 100$$

Where;
Ns: Average number of tumors in sample
Nc: Average number of tumors in negative control

iii. DNA Protection Assay

DNA protection assay was performed to study the effect of plant extracts on DNA by following the previously published protocol (Tian and Hua, 2005). Each reaction was conducted in an Eppendorf tube at a total volume of 12 µl containing 3 µl of 0.5 µg plasmid DNA (pBR322, Fermentas), 3 µl of 2 mM FeSO4 and 2 µl of test samples at various concentrations. After that 4 µl of 30% H_2O_2 was added and the mixture was kept at 37 °C for 1 hr. The mixture was employed to agarose gel electrophoresis followed by the staining of DNA bands. Quantification was done by scanning the intensity of bands with Quantity One programme (version 4.2.3, BioRad Co.).

RESULTS

Phytochemical evaluation

Phytochemical evaluation of various extracts of *S. lappa* revealed the presence of a wide variety of phytoconstituents as shown in Table 1.

Table 1: Phytochemical analysis of root extracts of *S. lappa.*

Phyto-chemicals	Results	Phyto-chemicals	Results
Alkaloids	+	Tannins	-
Lignans	+	Amino acid	+
Steroids	+	Glycosides	+
Flavonoids	+	Saponins	-
Anthraquinones	+	Sesqui-terpenes	+

(+); Presence of compounds, (-) Absence of compounds

Biological Activities

i. Antibacterial Assay

Root extracts of *S. lappa* exhibited significant antibacterial activity against tested bacterial strains (*S. aureus*, *E. coli* and *P. aeruginosa)*. Antibacterial potential was found to increase in direct proportion with increasing concentration of test extracts. In case of *S. aureus*, maximum activity was exhibited by CME and CEE, while CAE showed minimum antibacterial potential (Figure 2).

CAE showed highest antibacterial activity against *P. aeruginosa* while PEE was minimally active against this strain. Activities of CME, CEE and EAE were also significant (Figure 3). In case of *E. coli*, lowest bacterial inhibition potential was shown by CAE while CEE, CME, PEE and EAE showed almost same inhibition values (Figure 4).

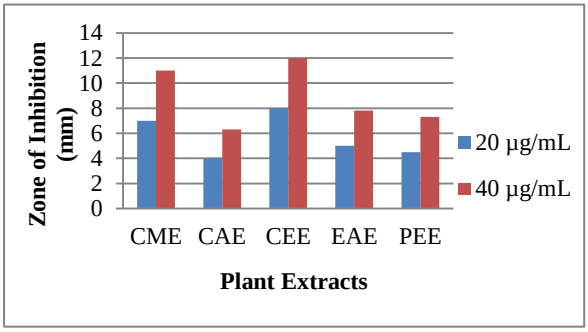

Figure 2: Anti-bacterial activity of various extracts of *S. lappa* against *S. aureus*.

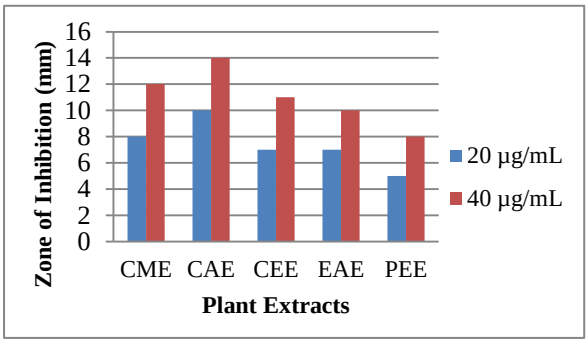

Figure 3: Anti-bacterial activity of various extracts of *S. lappa* against *P. aeruginosa*.

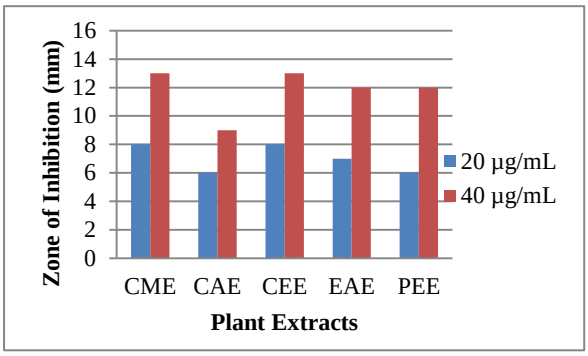

Figure 4: Anti-bacterial activity of various extracts of *S. lappa* against *E. coli*.

To determine the possible class of phytochemicals attributable for antibacterial activity we developed a correlation with different constituents that were found to be present in crude extracts of *S. lappa*. Flavonoids were found to have the best correlation (r=0.527) with antibacterial potential of *S. lappa* extracts against *P. aeruginosa*.

ii. Antitumor Activity

Antitumor activity of root extracts of *Saussurea lappa* was determined by potato disc assay method. For this purpose, extracts with three different concentrations were used. The maximum anti-tumor activity was shown by CME and minimum by CAE. It was observed that the inhibition of tumor increases by increasing the concentration of root extract (Figure 5). A correlation was developed between antitumor potential and phytoconstituents that were found to be present in S. *lappa* extracts. Most significant value (r=0.857) was shown by terpenes, thus suggesting that most probable class of phytochemicals responsible for antitumor potential of *S. lappa* extracts are terpenes.

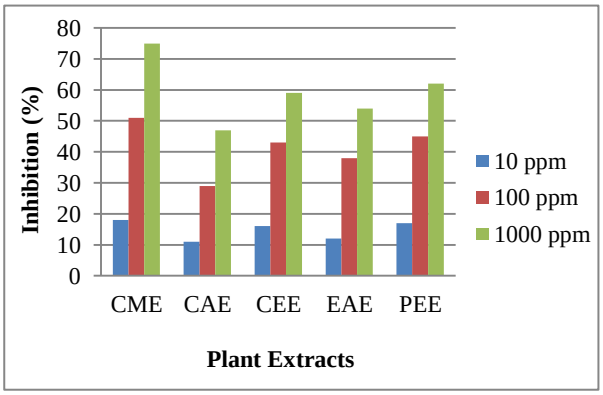

Figure 5: Anti-tumor activity of various extracts of *S. lappa* root.

iii. DNA Protection Assay

It was carried out on the basis of H_2O_2 and $FeSO_4$ photolysis in order to examine the DNA protection activity of root extracts of *S. lappa*. Maximum protection activity was observed by 100ppm concentration of methanolic and ethyl acetate extracts while ethanolic, petroleum ether and aqueous extracts showed less protection potential (Figure 6).

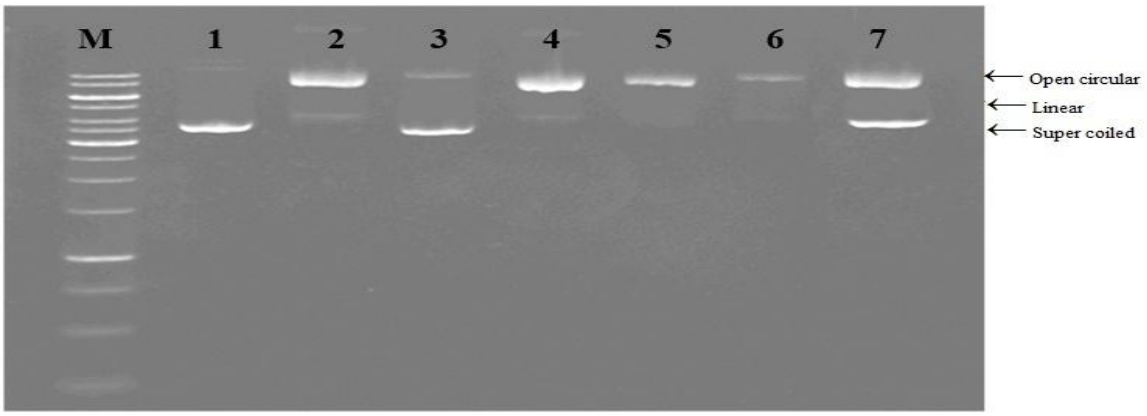

Figure 6: DNA protection assay. Lane M: 1kb DNA ladder, Lane 1: 100 ppm CME + 2 mM $FeSO_4$ + 30% H_2O_2 + pBR322, Lane 2: 100 ppm CEE + 2 mM $FeSO_4$ + 30% H_2O_2 + pBR322), Lane 3: 100 ppm EAE + 2 Mm $FeSO_4$ + 30% H_2O_2 + pBR322), Lane 4: 100 ppm PEE + 2 Mm $FeSO_4$ + 30% H_2O_2 + pBR322), Lane 5: 100 ppm CAE + 2 Mm $FeSO_4$ + 30% H_2O_2 + µL pBR322, Lane 6: plasmid DNA + 30% H_2O_2, Lane 7: plasmid DNA (pBR322).

DISCUSSION

Since the existence of human being on earth, plants are being used for food and medicine as well. Nature has gifted plants with various potentials which are beneficial for their users. The need of the hour is to screen a number of medicinal plants for promising biological activities in order to design novel drugs for the treatment of different diseases. The phytoconstituents evaluation of the root of *S. lappa* indicated the presence of alkaloid, flavonoids, glycosides sesquiterpenes, anthraquinones, lignans and steroids. However, saponins and tannins were not detected in current study.

Phytoconstituents possess therapeutic potentials, for example, the presence of flavonoids and resins are attributable for anti-inflammatory activity (Chandur et al., 2011). Phenolics are important class of phytoconstituents including flavonoids and tannins, that possess antioxidant potentials (Okamura et al., 1993; Rice-Evans et al., 1996; Kähkönen et al., 1999). Since these compounds were found in extracts under study that might be responsible for the potent antioxidant activity of *S. lappa* in current research.

Antibacterial activity was tested against two gram-negative strains (*E. coli* and *P. aeruginosa*) and one gram positive-strain (*S. aureus*). CEE showed maximum inhibition against the *S. aureus*, CAE showed maximum zone of inhibition against *P. aeruginosa*, while CME, CEE, EAE and PEE showed about 90% antibacterial activity against *E. coli*. It has been revealed that flavonoids can impart antibacterial impact by preventing nucleic acid synthesis and cytoplasmic membrane function (Goswami and Chatterjee, 2014; Xie et al., 2015). Flavonoids were detected in different extracts of subject plant therefore antibacterial potential of *S. lappa* might be attributed to the presence of flavonoids.

Anti-tumor activity of root extracts of *Saussurea lappa* was determined by potato disc assay method. Different concentrations of crude extracts were employed to check tumor inhibition potential that showed different percentage of tumor inhibition. Percentage inhibition of tumor was found in direct proportion to the concentration of root extracts of *S. lappa.* Twenty percent inhibition of tumor is considered as minimum significant value for anti-tumor potential of plant extract. Crude methanolic extract 1000 ppm showed maximum inhibition i.e. 75.86%. It was followed by 1000 ppm petroleum ether extract (PEE) showing 61 % inhibition, while 1000 ppm ethanolic and ethyl acetate extracts showed 54.37% and 57.82 % inhibition respectively. It is

obvious that tumor formation occurs due to imbalance between cell proliferation and cell death, which is maintained through regulation of various signal transduction pathways. Active cell death or programmed cell death (apoptosis) is caused by various physiologic or non-physiologic cell injuries including DNA damage (Folkman, 1971; Vogelstein et al., 1988). On the basis of results from preliminary studies, a cytotoxic herb *Saussurea lappa* was chosen to examine its cytotoxic effects that showed promising cytotoxic potential.

Methanolic and ethyl acetate extracts of subject plant showed promising DNA protection potential. Cells are susceptible to attack by reactive oxygen species (ROS) that are generated by both endogenous metabolisms and exogenous resources (Lenzen, 2008). Excessive production of ROS leads to oxidative stress resulting in severe pathological impacts on important bio macromolecules such as DNA and proteins. Although, cells adopt various compensatory mechanisms to counteract the deleterious impacts of oxidative stress but in some conditions, DNA damage cannot be prevented and eventually correlate with etiology, resulting in different diseases such as cancer (Birben et al., 2012). Polyphenols possess antioxidant potential and protect cells against hazardous impacts of free radicals. They can also block the action of enzymes responsible for cancer development as well as deactivate substances that promote the growth of cancers (Goswami and Chatterjee, 2014). A wide variety of polyphenols was detected in *S. lappa* that might be attributable for DNA protection potential.

CONCLUSION

The undertaken study illustrates that *S. lappa* is an enriched reservoir of phytoconstituents possessing antitumor, antibacterial and DNA protection potential. The preliminary study further demands bioassay guided isolation of compounds of interest from subject plant which are responsible for the aforementioned activities.

Conflicts of Interest

The authors declare no conflict of interest

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