RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.2.262-268

Phytochemical analysis and biosynthesis of silver nanoparticles from aqueous extract of seeds of *Sesamum indicum*#

V. Keerthika[1*], A. R. Nisha[2], Suresh N Nair[3], Preethy John[3], V. Ramnath[4], R. Shankar[1] and Arya Mohan[1]

Department of Veterinary Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651.
Kerala Veterinary and Animal Sciences University, Kerala, India.

Citation: Keerthika, V., Nisha, A.R., Suresh, N. N., Preethy, J., Ramnath, V., Shankar, R. and Arya, M. 2022. Phytochemical analysis and biosynthesis of silver nanoparticles from aqueous extract of seeds of *Sesamum indicum*. *J. Vet. Anim. Sci.* **53**(2): 262-268
DOI: https://doi.org/10.51966/jvas.2022.53.2.

Received: 07.11.2021 *Accepted: 11.12.2021* *Published: 30.06.2022*

Abstract

Plants have been in use in medicine from time immemorial. Sesamum indicum, an ancient seed crop, has been widely used mainly for its oil. It is regarded as highly beneficial for its nutritive value and therapeutic effect. It is known to have anti-allergic, anti-inflammatory, anti-cancer, analgesic and many other pharmacological activities. In this study, seeds of S. indicum were subjected to aqueous extraction followed by evaluation for the presence of phytochemical constituents. The aqueous extract of seeds of S. indicum after phytochemical testing, was found to contain alkaloids, phenols, steroids, flavonoids, diterpenes, glycosides and tannins. Following this, silver nanoparticles were biosynthesised from the extract, where the phytochemicals acted as reducing and capping agents for the nanoparticles formed. The synthesised nanoparticles were characterised by using UV-visible spectroscopy, energy dispersive X-ray spectroscopy, field emission scanning electron microscopy and X-ray diffraction. The synthesis of nanoparticles from plant extract is gaining importance now-a-days owing to the effectual activity elicited by them when compared to activity of plant compounds alone.

Keywords: *Sesamum indicum, phytochemicals, silver nanoparticles*

Traditional medicines, which contain compounds derived from medicinal plants, are used by approximately 80 per cent of people in developed countries (Yadav and Agarwal, 2011). Medicinal plants are a valuable source of bioactive compounds with a variety of activities. Secondary plant metabolites (phytochemicals), as they are commonly known, have been linked to a variety

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. *MVSc Scholar*
2. *Assistant Professor and Head*
3. *Assistant Professor*
4. *Professor and Head, Department of Veterinary Physiology*

**Corresponding author: keerthikavivekanandan@gmail.com, Ph: 9944051953*

of biological properties that protect against a range of diseases (Renjith and Sankar, 2020).

Sesame (*Sesamum indicum*), one of the world's important oil crop, is a member of Pedaliaceae family. It is an annual shrub with white bell-shaped flowers with a hint of blue, red or yellow with or without branches. It is grown for the production of seeds that are rich in oil content. These seeds are known to have desirable pharmacological effects. Sesame seeds are proven for their antioxidant activity (Anilakumar *et al*., 2010). The protein from sesame possesses antihyperlipidemic property which reduces triacylglycerol and cholesterol levels in plasma (Biswas *et al*., 2010). The antimicrobial activity of sesame has been reported by Saleem, 2011 against *Salmonella typhi*. Anticancer activity has been observed with myrsitic acid present in sesame seeds (Shasmitha, 2015). Sesamin from sesame has been known to have antihypertensive action in animals (Nakano *et al*., 2006). Miscellaneous effects like antipyretic, anti-inflammatory, wound healing, analgesic activities have been reported (Mushtaq *et al*., 2020).

Irrespective of the pharmacological effects that bioactive plant components possess, their use in the medicinal field has a long way to go. Exhaustive research has been conducted to facilitate nano delivery of these phyto-constituents. These nano formulations are known to have increased bioavailability, solubility, achieving determined target with reduce dose levels (Subramanian *et al*., 2016). Silver nanoparticles play an important role in the field of nanotechnology because of their extraordinary properties including chemical stability, conductivity, catalytic activity and biological effect such as antibacterial (Qais *et al*., 2019), antifungal (Ghojavand *et al*., 2020), antiviral (Sujitha *et al*., 2015), anti-inflammatory (Moldovan *et al*., 2017), anti-oxidant (Keshari *et al*., 2020), anti-plasmodial, anti- diabetic (Kuppusamy *et al*., 2016) and anticancer (Basak *et al*., 2018) activities. They are also known to have larvicidal, acaricidal wound healing properties and used for the treatment of water (Firdhouse and Lalitha, 2015). The present study focused on the production of AgNPs from the aqueous seed extract of *Sesamum indicum* (*S. indicum*) and their characterisation.

Materials and methods

Collection of seeds and identification

The seeds of *S. indicum* were collected from Avalpoondurai, Erode, Tamilnadu (Fig. 1). The plant along with seeds were authenticated from Research and PG Department of Botany St. Thomas College, Thrissur (Fig. 2).

Fig. 1. Seeds of *Sesamum indicum*

Fig. 2. Herbarium

Aqueous extract from seeds of S. indicum

The seeds of *S. indicum* were cleaned, dried and sieved. Then they were crushed and defatted using hexane as a solvent. The defatted residue was then allowed for air drying and kept in Soxhlet apparatus for hot aqueous extraction. Subsequently, it was allowed for drying in the rotary evaporator. The yield of extract was calculated from the extract obtained (Aathira *et al*., 2021). Finally, the resultant product was stored in refrigerator for further study.

Phytochemicals analysis

The secondary metabolites like alkaloids, phenols, steroids, flavonoids, diterpenes, triterpenes and saponins from the aqueous extract of seeds of *S. indicum* was examined as per method described by Harborne (1998).

For detecting alkaloids, one gram of the extract was mixed with 5 mL of ammonia before being extracted with the same volume of chloroform. Five millilitres of dilute hydrochloric acid were added to this extract. The obtained acid layer was then tested for the presence of alkaloids by using various reagents as described below.

Mayer's test - Eight drops of Mayer's reagent were added to 1 mL of acid layer. The presence of alkaloids was indicated by the formation of a cream-colored precipitate.

Dragendorff's test - One mL of acid extract was mixed with eight drops of Dragendorff's reagent. The presence of alkaloids was indicated by the formation of a reddish-brown precipitate.

Hager's test - Eight drops of Hager's reagent were added to 1 mL of acid extract. The presence of alkaloids was indicated by the formation of yellow precipitate.

Wagner's test – Equal amount of extract and Wagner's reagent were mixed together. The presence of alkaloids was indicated by the formation of a reddish-brown precipitate.

The presence of steroids was detected using Salkowski's test, which includes adding 3 mL of chloroform to 50 milligrams of the extract and dissolving it. The solution was then allowed to stand after adding few drops of concentrated sulphuric acid. The presence of steroids was indicated by the formation of a red colour. Also analysed by Liebermann Burchardt test by adding 3 mL of chloroform with 50 mg of extract and mixed. Along the sides of the test tube, five drops of acetic anhydride and one millilitre of concentrated sulfuric acid were added. The presence of steroids was confirmed by the formation of a reddish ring at the junction of two layers.

The detection of glycosides was done by Benedict's test by mixing the extract (50 mg) with 1 mL of water before adding 5 mL of Benedict's reagent. The presence of reducing sugars was indicated by the formation of brown or red precipitate.

The detection of phenols was performed using ferric chloride test by adding five milligrams of the extract to one millilitre of water, dissolving it and adding five drops of 10% ferric chloride. The appearance of a bluish black colour indicated the presence of phenols.

The presence of tannins was confirmed by ferric chloride test by adding two milligrams of extract to three millilitres of one per cent ferric chloride solution. The presence of tannins was indicated by the development of a blue-black or brownish green coloration.

The flavonoids were detected by ferric chloride test by adding four drops of neutral ferric chloride solution to 2 mL of the methanol extract (0.5-gram extract in 10 mL methanol). The presence of flavonoids was indicated by the formation of green colour.

The diterpenes in the extract were detected by adding five milligrams of extract with three millilitres of 5 per cent copper acetate solution. The presence of diterpenes was depicted by the formation of green colour.

The triterpenes were detected using Salkowski test, which includes mixing three milligrams of extract with three millilitres of chloroform and shaken with three millilitres of concentrated sulphuric acid. The presence of triterpenes was indicated by the development of yellow colour in the lower layer after standing.

The presence of saponins were done by froth test by adding 5 mL of water to 200 mg of extract and the tube was shaken. It was identified by the persistence of foam produced for 10 min.

Biosynthesis of silver nanoparticle from aqueous extract

The silver nanoparticles were synthesised by mixing 10 mL of 1 mM silver

nitrate solution with 10 mL of 2 grams of aqueous extract, dissolved in deionised water. It was kept on hot plate and the extract was added under continuous stirring. Then the solution was kept under bright sunlight for the colour change manifesting the development of nanoparticles.

Characterisation of the synthesised silver nanoparticles

The synthesised silver nanoparticles were characterised by UV- visible spectroscopy (Perkin-Elmer, Lamda 25) with wavelength of 300-700 nm. The silver nanoparticles from the plant were examined under field emission scanning electron microscope at Amrita Centre for Nanosciences and Molecular Medicine, Kochi. Using ImageJ software, the size of the nanoparticles was measured.
The energy dispersive X-ray spectroscopy for the biosynthesised silver nanoparticle was performed to detect the presence of specific elements at Amrita Centre for Nanosciences and Molecular Medicine, Kochi.

The phase analysis for the biosynthesised silver nanoparticles was done using X-ray diffraction spectroscopy at Centre for Materials for Electronics Technology (C-MET) Athani, Thrissur. They were verified at 30 kV voltage, 20 mA current at a rate of 10°2θ/ min with CuKα radiation.

Results and discussion

Phytochemical analysis

The yield of the extract was calculated as 13.63 percent**.** Similar extract yield of 13.37 per cent has been reported from aqueous extract of sesame seeds (Hussain *et al*., 2018). The aqueous extract from the seeds showed the presence of steroids, alkaloids, glycosides, flavonoids, phenols, diterpenes and tannins. Phytochemicals analysis by Narasimhan and Mohan (2012) from hexane and methanolic extract of seeds of sesame revealed presence of alkaloids, polyphenols, glycosides, flavanoids, carbohydrates, proteins, phytosterols and terpenoids. Ghani *et al*. (2012) examined the ethanolic defatted extract of *Sesamum indicum* and found to have alkaloids, tannins, phenols, glycosides, saponins, coumarins and flavonoids. These compounds exhibited anticancer activity in Human larynx epidermoid carcinoma (Hep-2 cell line), murine Mammary Adenocarcinoma (AMN-3) and human Rhabdomyosarcoma (RD). This is also in accordance with the work done by Usha *et al*. (2013) from the hot aqueous extract of seeds of *Butea monosperma*.

Biosynthesis of silver nanoparticles from aqueous extract

The manifestation of colour change was analysed by exposing the solution to sunlight that revealed the colour change for yellow to dark red solution (Fig. 3). This indicated the formation of silver nanoparticles. Alfuraydi *et al*. (2019) described similar colour change reaction with silver nanoparticles from sesame oil cake and determined that the transition was caused by surface plasmon resonance and free electron modifications. The aqueous extract containing phytochemicals reduced the silver nitrate to metallic silver and acted as reducing and capping agents for the nanoparticles produced as reported by Basu *et al*. (2016).

Fig. 3. Colour change from yellow to dark red

Characterisation of silver nanoparticles from aqueous extract

The silver nanoparticles from the sesame seeds were analysed using UV-visible spectrophotometer. It showed peak of absorption at 432 nm (Fig. 4). Similar peaks have been observed with Meena and Chouhan (2015). They described that dark red colour was due to the excitation of electrons resulting from the surface plasmon resonance when analysing the fenugreek seed aqueous extract. Azizi *et al*. (2013) also noticed peak at 420 nm with silver

nanoparticles prepared from aqueous extract of *Sargassum muticum*.

The micrographs of FESEM were used to determine the size of the nanoparticle, which was biosynthesised from sesame seeds. They showed a range of 14 - 48 nm in nano scale (Fig. 5). This has been measured using ImageJ software with 64-bit JAVA. The analysis indicated the spherical nanoparticles in solitary and cluster form. This result was similar to consistent silver nanoparticles from *Delphinium denudatum* root produced by Suresh *et al*. (2014), where the FESEM analysis exhibited the nanoparticles in spherical shape. Benakashani *et al*. (2016) also obtained similar spherical nanoparticle of 10 to 40 nm size.

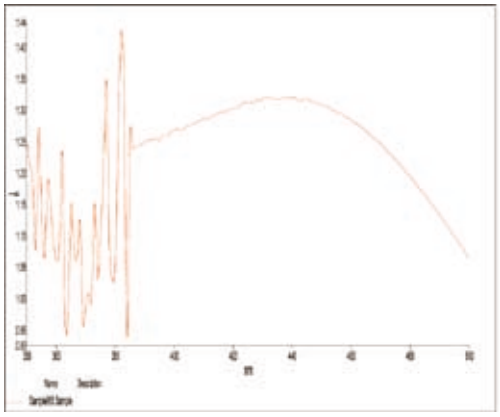

Fig. 4. Absorption spectra for S-AgNPs

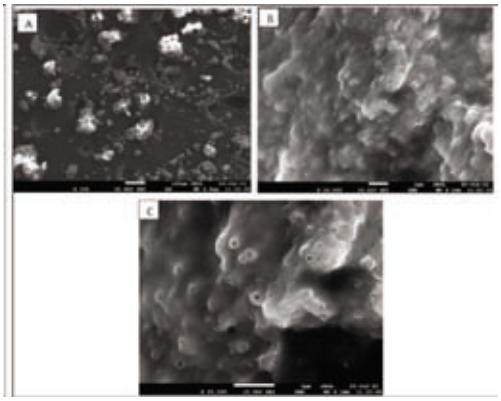

Fig. 5. FESEM images for S-AgNPs- A-100X, B- 10,000 X and C- 20,000 X magnification

With the energy dispersive X-ray spectroscopy, the main peak observed in the graph was silver. Miscellaneous peaks were noticed along the peak of silver. The other peaks were carbon, sulphur, chlorine and oxygen. This might be acquired from the plant extract (Fig. 6 and 7). The findings of this study were in accordance with Bhagat *et al*. (2019).

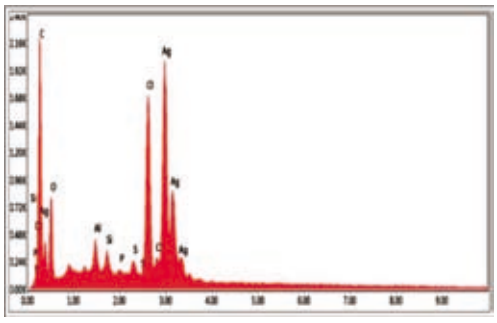

Fig. 6. EDS analysis of S-AgNPs

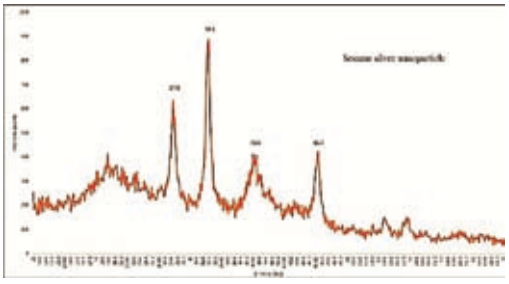

Fig. 7. Diffraction peak for S-AgNPs

With X-ray diffraction analysis, the diffraction analysis for silver nanoparticles produced distinct peaks at 27.8 °, 32.3 °, 38.4 ° and 46.3°. Similar peaks were seen with silver nanoparticles from *S. indicum* oil cake as done by Alfuraydi *et al*. (2019). He also added that those nanoparticles from sesame oil cake were analysed to have better antimicrobial and anti-tumour activity. Miscellaneous peaks with peaks of silver nanoparticles were due to presence of phytochemicals from the seed extract (Ibrahim, 2015).

Conclusion

The aqueous extract from seeds of *S. indicum* analysed for phytochemicals (steroids, alkaloids, glycosides, flavonoids, phenols, diterpenes and tannins) is known to have many pharmacological activities like anti-cancer, anti-inflammatory, anxiolytic, thrombolytic actions. The silver nanoparticles prepared from the aqueous extract was characterised by using UV-visible spectroscopy, energy dispersive X-ray spectroscopy, field emission scanning electron microscopy and X-ray diffraction. Characterisation of nanoparticles is imperative

in revealing their diverse biological activities. These silver nanoparticles biosynthesised from *S. indicum* could be evaluated for several enhanced pharmacological and therapeutic effects that would be beneficial to the population.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aathira, K. K., Bibu, J. K., Dhanusha, G., Haima, J. S., Sujith, S., Shynu, M. and Nisha, A. R. 2021. Qualitative and quantitative analysis (GC-MS) of methanol extract of *Crataeva nurvala* stem bark. *J. Vet. Anim. Sci.* **52:** 135-141.

Alfuraydi, A.A., Devanesan, S., Al-Ansari, M., AlSalhi, M.S. and Ranjitsingh, A.J. 2019. Eco-friendly green synthesis of silver nanoparticles from the sesame oil cake and its potential anticancer and antimicrobial activities. *J. Photochem. Photobiol.* **192:** 83-89.

Anilakumar, K.R., Pal, A., Khanum, F. and Bawa, A.S. 2010. Nutritional, medicinal and industrial uses of sesame (*Sesamum indicum* L.) seeds-an overview. *Agric. Conspec. Sci.* **75:** 159-168.

Azizi, S., Namvar, F., Mahdavi, M., Ahmad, M.B. and Mohamad, R. 2013. Biosynthesis of silver nanoparticles using brown marine macroalga, *Sargassum muticum* aqueous extract. *Mater.* **6:** 5942-5950.

Basak, P., Majumder, R., Jasu, A., Paul, S. and Biswas, S. 2018. Potential Therapeutic Activity of Bio-Synthesized Silver Nanoparticles as Anticancer and Antimicrobial Agent. In *IOP Conference Series: Materials Science and Engineering;* September, 2018, p. 012020.

Basu, S., Maji, P. and Ganguly, J. 2016. Rapid green synthesis of silver nanoparticles by aqueous extract of seeds of *Nyctanthes arbor-tristis*. *Appl. Nanosci.* **6:** 1-5.

Benakashani, F., Allafchian, A.R. and Jalali, S.A.H. 2016. Biosynthesis of silver nanoparticles using *Capparis spinosa* L. leaf extract and their antibacterial activity. *Karbala Int. J. Mod. Sci.* **2:** 251-258.

Bhagat, M., Anand, R., Datt, R., Gupta, V. and Arya, S. 2019. Green synthesis of silver nanoparticles using aqueous extract of *Rosa brunonii* Lindl and their morphological, biological and photocatalytic characterizations. *J. Inorg. Organomet. Polym. Mater.* **29:** 1039-1047.

Biswas, A., Dhar, P. and Ghosh, S. 2010. Antihyperlipidemic effect of sesame (*Sesamum indicum* L.) protein isolate in rats fed a normal and high cholesterol diet. *J. Food Sci.* **75:** 274- 279.

Firdhouse, M.J. and Lalitha, P. 2015. Biosynthesis of silver nanoparticles and its applications. *J. Nanotechnol.* **2015: 1-15**.

Ghani, N.A., Umran, M.A. and Shawkat, M.S. 2012. Cytotoxic activity of ethanol extract of *Sesamum indicum* seeds to cancer cell lines *in vitro*. *Iraqi J. Med.* Sci. **5:** 28-33.

Ghojavand, S., Madani, M. and Karimi, J. 2020. Green synthesis, characterization and antifungal activity of silver nanoparticles using stems and flowers of felty germander. *J. Inorg. Organomet. Polym. Mater.* **30:** 2987-2997.

Harborne, J.B. 1998. Phytochemical Methods: A guide to the modern techniques of plant analysis. (3rd Ed.) Chapman and Hall, London. 320p.

Hussain, S.A., Hameed, A., Ajmal, I., Nosheen, S., Suleria, H.A.R. and Song, Y. 2018. Effects of sesame seed extract as a natural antioxidant on the oxidative stability of sunflower oil. *J. Food Sci. Technol.* **55:** 4099-4110.

Ibrahim, H.M. 2015. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *J. Radiat. Res. Appl. Sci.* **8:** 265-275.

Keshari, A.K., Srivastava, R., Singh, P., Yadav, V.B. and Nath, G. 2020. Antioxidant and antibacterial activity of silver

nanoparticles synthesized by *Cestrum nocturnum*. *J. Ayurveda. Integr. Med.* **11:** 37-44.

Kuppusamy, P., Yusoff, M.M., Maniam, G.P. and Govindan, N. 2016. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications–An updated report. *Saudi Pharm. J.* **24:** 473-484.

Meena, R.K. and Chouhan, N. 2015. Biosynthesis of silver nanoparticles from plant (fenugreek seeds) reducing method and their optical properties. *Res. J. Recent Sci.* **4:** 1-5.

Moldovan, B., David, L., Vulcu, A., Olenic, L., Perde-Schrepler, M., Fischer-Fodor, E., Baldea, I., Clichici, S. and Filip, G.A. 2017. *In vitro* and *in vivo* anti-inflammatory properties of green synthesized silver nanoparticles using *Viburnum opulus* L. fruits extract. *Mater. Sci. Eng. C* **79:** 720-727.

Mushtaq, A., Hanif, M.A., Ayub, M.A., Bhatti, I.A. and Jilani, M.I. 2020. Sesame. In *Medicinal Plants of South Asia.* 601-615.

Nakano, D., Kwak, C.-J., Fujii, K., Ikemura, K., Satake, A., Ohkita, M., Takaoka, M., Ono, Y., Nakai, M. and Tomimori, N. 2006. Sesamin metabolites induce an endothelial nitric oxide-dependent vasorelaxation through their antioxidative property independent mechanisms: possible involvement of the metabolites in the antihypertensive effect of sesamin. *J. Pharmacol. Exp. Ther.* **318:** 328- 335.

Narasimhan, R. and Mohan, A. 2012. Phytochemical screening of *Sesamum indicum* seed extract. *Int. J. Pharm. Pharm. Sci.* **1:** 1298-1308.

Qais, F.A., Shafiq, A., Khan, H.M., Husain, F.M., Khan, R.A., Alenazi, B., Alsalme, A. and Ahmad, I. 2019. Antibacterial effect of silver nanoparticles synthesized using *Murraya koenigii* (L.) against multidrug-resistant pathogens. *Bioinorg. Chem. Appl.* **2019:** 1-11.

Renjith, M.R.D. and Sankar, M. 2020. Scope of phytochemicals in the management of covid-19. *Pharm. Res*. **3:** 26-29.

Saleem, T.M. 2011. Anti-microbial activity of sesame oil. *Int. J. res. phytochem. pharmacol.* **1**: 21-23.

Shasmitha, R. 2015. Health benefits of *Sesamum indicum*: a short review. *Asian J. Pharm. Clin. Res.* **8:** 1-3.

Subramanian, A.P., Jaganathan, S.K., Manikandan, A., Pandiaraj, K.N., Gomathi, N. and Supriyanto, E. 2016. Recent trends in nano-based drug delivery systems for efficient delivery of phytochemicals in chemotherapy. *RSC Adv.* **6:** 48294-48314.

Sujitha, V., Murugan, K., Paulpandi, M., Panneerselvam, C., Suresh, U., Roni, M., Nicoletti, M., Higuchi, A., Madhiyazhagan, P., Subramaniam, J. and Dinesh, D. 2015. Green-synthesized silver nanoparticles as a novel control tool against dengue virus (DEN-2) and its primary vector *Aedes aegypti*. *Parasitol. Res.* **114:** 3315-3325.

Suresh, G., Gunasekar, P.H., Kokila, D., Prabhu, D., Dinesh, D., Ravichandran, N., Ramesh, B., Koodalingam, A. and Siva, G.V. 2014. Green synthesis of silver nanoparticles using *Delphinium denudatum* root extract exhibits antibacterial and mosquito larvicidal activities. *Spectrochim. Acta A. Mol. Biomol. Spectrosc.* **127:** 61-66.

Usha, N.P., Dhanya, V.R. and Jose, S. 2013. *In vitro* efficacy of *Butea monosperma* against amphistomosis in cattle. *J. Vet. Anim. Sci.* **44:** 70-71.

Yadav, R.N.S. and Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *J. Phytol.* **3:** 10-14.

■