Insights into the phytochemical potential of Lawsonia inermis L. for future small molecule based therapeutic applications Vol. 11(1) pp. 1-7, June, 2020
Available online https://www.interesjournals.org/basic-clinical-studies.html
DOI: http:/dx.doi.org/10.14303/irjps.2020.006

Review Article

Insights into the phytochemical potential of *Lawsonia inermis* L. for future small molecule based therapeutic applications

Debapriya Das[1], Dipu Samanta[2], Rajat Banerjee[3], Suchita Sinha[1], Bidisha Mallick[1], Sayak Ganguli[4] & Debleena Roy[1]*

[1]Post Graduate Department of Botany, Lady Brabourne College, Kolkata 700017, India.

[2]Department of Botany, Dr. Kanailal Bhattacharyya College, Howrah, 711104, India.

[3]Dr. B. C. Guha Centre of Biotechnology and Genetic Engineering, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India.

[4]Department of Biotechnology, St. Xavier's College (Autonomous), Kolkata 700016, India."

*Corresponding author's e-mail: debleenaroy@rediffmail.com

Abstract

Lawsonia inermis L., commonly known as Henna plant, have been reported to be useful both medicinally and commercially. The phytochemical extract of this plant leaves have been reported to be a rich source of phenols, glycosides, anthraquinones and many other active constituents. Therapeutically, this plant has been used to treat diabetes, arthritis, obesity, ulcers, wounds, microbial infections, inflammation and liver damage. The extracts have also been instrumental in lowering blood sugar and cholesterol levels in mice. But one of the most crucial aspects of this plant is, limiting the growth of malignant cells. Extracts have shown apoptosis promoting activity in human cancer cell lines like breast cancer cells. The pigment lawsone, is commercially used on a large scale, as a dying agent for fabrics and skin. This component has shown potential role in reducing oxidative burst in cell, hence, establishing its role as an antioxidant, which should help researchers to manipulate the property, for establishing new potential drugs against cancer. Apart from "Lawsone" the small molecule reservoir of *Lawsonia inermis* L. have not been commercially utilized effectively and in the future these bioactive compound set should be explored for formulating new chemical entities.

Keywords: Antioxidant; Therapeutic; Small molecule; Drugs.

INTRODUCTION

Red henna or *Lawsonia inermis* L. (Lythraceae), is a perennial shrub, widely cultivated in tropical regions of Egypt, America, India and Middle East (Singh, et al., 2012), commonly known as Mehndi. Commonly known as Cypress shrub, Samphire, Mendika, Timir, Rakigarbha, Goranta, Kormi, Maruthani and Mayilanchi, this plant develops white and rose-red flowers. The plant grows to a height of about six meters. Leaves are opposite, sub-sessile, acuminate, lanceolate, glabrous and contain lawsone, (naphthoquinone) which is used to dye fingers, fabrics and hair. Henna fruits usually ripen at the end of summer, each fruit bearing about 40-45 seeds. When young, the plant has low dye content and lacks spine. As the plant matures, the dye content increases and spine formation occurs. In India, commercial Henna production is found in states of Rajasthan (Jodhpur and Pilani), Thane, Kalyan and Badlapur (Phirke, et al., 2013). In the Rajasthan region, due to an arid atmosphere, low rainfall and poor soil fertility conditions, cultivation of Henna plant by the farmers, provide a good source of income. Also, uninterrupted growth of Henna plant in elevated temperatures and poor soil conditions indicate the potential role of this plant in fixing atmospheric carbon at a time of climate crisis due to rise in global warming.

Henna plant leaves have a wide plethora of medicinal uses. Extracts of mainly leaves can be used as anti-diabetic (Widyawati, et al., 2019), anti-arthritis (Ramya, et al., 2015; Kadhem, 2016), in treatment of bacterial infections (Hussain, et al., 2011; Habbal, et al., 2011; Raja, et al., 2013; Rahiman, et al, 2013; Akintunde, et al., 2017), fungal infections (Rizvi, et al., 2013), diarrhea, obesity, liver damage (Bhaskaran and Shruthi, 2016; Mohamed, et al., 2016), ulcers (Goswami, et

al., 2011; Sravanthi, et al., 2011; Basipogu and Syed, 2015), gingivitis and stimulation of immune system (Uthayakumar, et al., 2014), when taken orally. There have also been reports that extract of seeds of this plant has potential antioxidant capacity (Chaibi, et al., 2017) and plant extract has helped in reducing proliferative growth of human cancer cell lines (Eldrini, et al., 2007). The extracts have also successfully tackled oxidative burst in cells. This antioxidant property was mainly due to the high polyphenolic content of the extracts (Uma, et al., 2010; Zohourian, et al., 2011). Commercial exploitation of phytochemicals from Henna plant in treating ailments has put this plant on high demand. Hence, there arises a need for mass scale Henna plant cultivation. Apart from "Lawsone" the small molecule reservoir of *Lawsonia inermis* L. have not been commercially utilized effectively. The bioactive compound set should be explored for formulating new chemical entities for future use.

Medicinal importance

Anti-carcinogenic properties

The effects of leaf extracts of *Lawsonia inermis* when tested on various cancer cell lines to assess the anti-carcinogenic effects, showed significant cytotoxic effects. The extracts slowed down the tumour growth of the breast cancer and colon cancer cell lines, but no effects were seen against liver cancer cell lines (Eldrini, et al., 2007). *Lawsonia* leaf extracts were reported to be a free radical scavenger, which could reduce the oxidative stress in serum, kidney and liver of Wister albino rats. The activity of hydrogen peroxidase, lipid peroxide in serum and tissue homogenates was found to be high with rats treated with high dose of henna extract. There was a significant decline in catalase activity (Al-Damegh, 2014). It was seen that *Lawsonia* extracts showed effective DPPH radical scavenging activities. It also showed lipid peroxidation activities. Mice which were treated with *Lawsonia* extracts showed low levels of glucose, cholesterol, triacylglycerol and lipoprotein cholesterol in their blood (Ojewunmi, et al., 2013). This potential antioxidant activity was mainly attributed to the phytochemical constituent of the leaves, specially the high phenolic content.

Treating infections

The methanolic extracts of different plants were tested for their antibacterial activities, by disc diffusion method (Hussain, et al., 2011; Raja, et al., 2013). *Lawsonia inermis* showed antibacterial activity against *Escherichia coli, Bacillus cereus*, *Staphylococcus aureus* and many other strains. These effective activities against the bacterial strains are mainly due to flavonoids and glycosides, the main constituents of the leaves. Recent research has also suggested that lawsone, from the leaves of *Lawsonia inermis*, can be used externally due to its low toxicity. The extract of Henna leaves was also found to be effective on the strains of *Enterococcus*, *Micrococcus*, *Trueperella, Klebsiella*, *Proteus,* and *Shigella* (Akintunde, et al., 2017). Antibacterial and antifungal activities for Henna plants, growing *in vivo* (growing on soil) and *in vitro* (growing in tissue culture laboratory) showed no drastic difference in their activities against *C. albicans*, *S. aureus*, *P. aeruginosa*, *B. cereus*, *E. coli*, *A. niger*, *Trichoderma* sp. and *Fusarium* sp. (Rahiman, et al., 2013). Methanolic extracts of the leaves when evaluated showed potential activity against disease causing bacteria. Extracts also showed inhibition to the growth of fungus like, *Fusarium*, *Alternaria* and *Mucor* (Rizvi, et al., 2013). Potential antifungal action was found in the leaf extracts of the plant against clinical isolates of *Candida* sp. (Yigit, 2017). Apart from alcoholic extracts, petroleum ether extracts of the leaves were reported to possess antifungal activities against *Candida albicans*, *Saccharomyces cerevisiae*, *Trichophyton mentagrophytes, Trichophyton violaceum and Aspergillus flavus* (Suleiman and Mohamad, 2014). Lawsone (commercial) was treated with a base in the presence of water to yield naphthoquinone derivatives, which were effective on fungal and bacterial strains but showed less effects on strains of *Candida* sp (Rahmoun, et al., 2012). Convenient and low-cost metal nanoparticles were obtained, using leaf extracts of *Lawsonia inermis,* showed effective antibacterial properties (Naseem and Farukh, 2015). A comparison was drawn where the *in vitro* antifungal property of the chloroform, methanol and aqueous extracts of *Lawsonia* leaves showed potential action against *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypsum* and *Microsporum fulvum,* which are commonly involved in causing skin diseases (Sharma, et al., 2011). Certain plant products like alkaloids, steroids, flavonoids, terpenoids, and saponins, found in ethanol extract were accounted responsible for inhibiting bacterial growth. Hexane and ethyl-acetate extracts, rich in glycosides, showed potent antibacterial activity as well (Yusuf, 2016). Silver nanoparticles, coated with henna leaf extracts, evaluated for their antibacterial activity, successfully inhibited growth of bacteria, as seen by disc diffusion method (Kumar and Kathireswari, 2016). In a study, 63 patients (divided into 5 groups) suffering from gingivitis, a disease caused by formation of bacterial plaque eventually infect the underlying tissue, causing periodontitis, were asked to rinse with 3 different concentrations of *Lawsonia inermis* leaf extracts (50000 µg/ml, 10000 µg/ml and 5000 µg/ml). Leaf infusions having a concentration of 10000 µg/ml was found to be most effective in curing the infection (Zubardiah, et al., 2012).

Anti-ulcer effects

The antiulcer effects of water, chloroform and ethanolic extracts of leaves of *Lawsonia inermis* on rats with ulcers were tested (Goswami, et al., 2011). In case of aspirin induced ulcers, chloroform extracts showed a significant reduction of ulcers with an increase in dose. Volume of gastric juice, total acidity, ulcer index and free acidity were the parameters used to assess the ulcer conditions. Methanolic extracts of the leaves, showed effective results on ulcer induced rats, up to above 50% (Goswami, et al., 2011). Cold stress induced ulcers were also cured by ethanolic leaf extracts in Wistar rats. After phytochemical

screening, a large number of chemical compounds like alkaloids, glycosides, naphthoquinones, terpenoids, tannins, phenolic compounds and carbohydrates were identified which directly or indirectly helped to cure the ulcers (Sravanthi, et al., 2011).

Analgesic activity

Methanolic leaf extracts were seen to exhibit analgesic, anti-inflammatory and CNS depressant activities on mice. Analgesic activity was measured by using acetic acid-induced writhing model and formalin-induced licking and biting in mice. The CNS depressant activity was investigated and seen that a dose of 500 mg/kg body weight induced higher analgesic activity in mice affected with acetic acid induced pain. Also, the methanolic leaf extracts showed significant CNS depressant and anti-inflammatory activity in a dose dependant manner (Nesa, et al., 2014).

Antiarthritic effect

Rheumatic arthritis is an inflammatory, autoimmune illness. Aqueous extracts of *Lawsonia inermis* leaves at different concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) were used to assess the inhibition of protein denaturation and membrane lysis. The effects of leaf extracts were compared with diclofenac sodium drug. It was seen that the leaf extracts were successful in protective activity against arthritis due to the active constituents of the leaves (Ramya, et al., 2015).

Anti-diabetic property

The hypoglycaemic and antihyperlipidemic effects of leaf extracts were evaluated. For this purpose, mice, which were subjected to hyperglycaemic conditions were treated with 70% ethanolic extract of leaves of *Lawsonia*. The results showed that mice which were treated with leaf extracts showed normal glucose levels in their blood. It was also observed that triacylglycerols and cholesterols were low for mice which were treated with leaf extracts (Abdillah, et al., 2008). The extracts also showed inhibitory effects on the glucose utilization. It was seen that triacylglycerol accumulation was inhibited to a certain extent (Arayne, et al., 2007).

Hepatoprotective activity

Wistar albino rats were administered with paracetamol (750 mg/kg) to induce liver damage. When subjected to *Lawsonia inermis* leaf extracts (ethanolic), it was seen that a dose of 400 mg/kg lowered the bilirubin level and heightened the SGPT and total protein levels, exhibiting hepatoprotective activities (Bhaskaran and Shruthi, 2016). Liver damage in mice, induced by carbon tetrachloride treatments were also treated by *Lawsonia inermis* leaf extracts, when administered orally at a dose of 100 mg/kg and 200 mg/kg body weight. It was seen that, mice, which were administered with *Lawsonia* extracts, showed quick recovery from liver damage at a dose dependant manner (Mohamed, et al., 2016).

Immuno-stimulatory effects

Methanolic extracts of *Lawsonia inermis* leaves were administered in fishes, affected with ulcers. There was an increase in levels of red blood cells, white blood cells, haematocrit and haemoglobin. Mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration decreased. Thus, henna plant extracts were reported to have immunostimulatory functions (Uthayakumar, et al., 2014).

Chemical analysis of leaf extract of Henna

High performance liquid chromatography (HPLC) has been a very reliable method in reporting the phytochemical constituents of plant extract. Chemical composition of the extract of leaves of Henna depends on the conditions of extraction and the solvent used. Microwave assisted extraction reported a higher level of phenol and antioxidant potential, compared to those extracted at normal room temperature (Zohourian, et al., 2011). Factors like temperature, time, acetone concentrations are necessary parameters that regulate proper phenol extraction from leaves (Uma, et al., 2010). It was reported that a temperature of 39.57ºC, time of 73.78 minutes and acetone concentration of 48.07% resulted in optimum phenol extraction (7203.74 mg GAE/100g of dry weight). Phytochemical screening of the extracts, not only showed high phenols, but also proved to have flavonoids, alkaloids, tannins and anthraquinones. All these chemicals impart antibacterial, antioxidant and cytotoxic property to the extracts (Mansoor, et al., 2016). ß-sitosterol, reported from the leaves also contained potential antioxidant capacity (Badhai, et al., 2016). A study reported that the extracts helped to stop the growth of human breast cancer cells (Omran, et al., 2017). Silica gel column chromatography identified a new compound with powerful antioxidant potential. This compound was further purified and characterized by spectroscopic methods as 1,2,4-trihydroxynapthalene-2-O-ß-D-glucopyranoside (Dhouafli, et al., 2017). Three other compounds, lalioside (2, 3, 4,6-tetrahydroxyacetophenone-2-O-ß-D-glucopyranoside), luteolin-7-O-ß-D-glucopyranoside and lawsoniaside (1,2,4-trihydroxynaphthalene-1,4-di-O-ß-D-glucopyranoside) were also reported in *L. inermis.* The antioxidant activity of these compounds was evaluated by DPPH and ß-carotene assays (Hsouna, et al., 2010). The various polyphenols, antioxidant, anti-proliferative and antigenotoxic effects of polar and non-polar extracts were analyzed (Kumar, et al., 2014). The extracts were seen to reduce the mutagenic effects of mutagens like 4-nitroquinoline1- oxide and nitrofurantoin and protected DNA damage against strong oxidants. The extracts were effective against the growth of cancer cell lines, like PC3 and Colo 205. Partial purification of the phenolics and flavonoids was done by adsorption chromatography, using a silica gel column. The phenolics and flavonoids were estimated using gallic acid and quercetin as standards. The antioxidant potential was measured by ABTS assay.

Lawsonia inermis L. plant extract was reported to have potent antioxidant properties (Saeed, et al., 2013). Extracts made with various solvents were also evaluated for their phenolic contents and radical scavenging properties. It was seen that, ethanolic preparations showed high antioxidant capacities.

The chemical space of Bioactive compounds in *Lawsonia inermis* L. and their potential use in Computer Aided Drug Discovery

Half of the world's repertoire of approved drugs over the last 30 years have come either directly from natural products or are derivatives of the original bioactive small molecule of plant origin. From the 1940s to date, of the 175 small molecules approved as drugs, 85 actually have natural origin. The history of plants towards treatment of myriad diseases is long and illustrious. However, internationally the rate of approval of new drugs has slowed down considerably. Despite technological advances in drug discovery, between 1996 and 2007, the number of new molecular entities approved by the US FDA has fallen from 53 to 17 per year—the same rate as over 50 years ago (FitzGerald, 2008; Munos, 2009). This trend has been attributed to the following factors:

1. The most acceptable hypothesis involves the overutilization of the "lowest hanging fruits" in terms of small molecule drug candidates that have been extensively investigated, and computational challenges hinder extension of traditional methods to more complex structures. Researchers refer to "rediscovering the sweet spot" as the discovery process (Brown and Superti-Furga, 2003), which have the potential to produce targeted screening libraries that ideate the anticipated characteristics of lead compounds (Welsch et al., 2010; Cheng, et al., 2012).

2. The second problem lies with the complexity of the diseases and the failure to identify suitable drug targets. Thus, making the process of prediction lead compounds difficult (Ramsay, et al., 2018).

3. Clinical trials involving model organisms often do not provide suitable results due to inter-species variations that are crucial to therapeutic action, thus making the process of drug discovery difficult (Hunter, 2008; Ehret, et al., 2017).

The Small Molecule Reservoir

It has reported that, 80% the plant derived drugs have established ethnopharmacological literature sources. Workers have indicated that co interactions can actually increase the potency of plant crude extracts and has attributed the reduction in efficiency of isolated and purified plant products as a result of this lack of co interactions (Lila and Raskin, 2005). As we are all aware that additive and synergistic effects results in potentiation, in which a group of compounds in a mixture interact to provide a combined effect that is equal to the sum of the effects of the individual components (additive) or where combinations of bioactive substances exert effects that are greater than the sum of individual components (synergistic) (Veeresham, 2012). Since *Lawsonia* posses a large repertoire of active principles, such as quinones, phenylpropanoids, flavonoids, terpenoids, phenolics, tannins, alkaloids, xanthones, coumarin, glucosides, naphthoquinone, saponins, triterpenoids, sterols and dioxin derivatives; it is a rich source for such potentiation and natural product-based drug discovery studies (Table I). Some identified small molecules such as isoplumpagin (a naphthoquinone from bark), lupeol, 30-norlupan-3-ol-20-one, betuhennan, betuhennanic acid and n-tridecanoate (bark), phenolic glycosides, lawsoniaside, β-sitosterol and stigmasterol (leaves) have been reported from Mehndi/ Henna plant along with 24-beta ethyl cholest-4-en-3-beta-ol from roots. (Singh and Luqman, 2014).

Table I. Phytochemicals identified from *Lawsonia inermis* L. leaf extracts.

Phytochemical	Formula	Molecular weight (g/mol)	Amount present (%)
1. Lawsone (Mohamed, et al., 2016)	$C_{10}H_6O_3$	174.15	0.5-1.0
2. Gallic acid (Bhaskaran and Suruthi, 2016)	$C_7H_6O_5$	170.12	0.24-0.72
3. Catechin (Bhaskaran and Shruthi, 2016)	$C_{15}H_{14}O_6$	290.26	0.76-1.50
4. Chlorogenic acid (Bhaskaran and Shruthi, 2016)	$C_{16}H_{18}O_9$	354.31	1.21-2.20
5. Epicatechin (Bhaskaran and Shruthi, 2016)	$C_{15}H_{14}O_6$	290.26	0.03-0.22
6. Caffeic acid (Bhaskaran and Shruthi, 2016)	$C_9H_8O_4$	180.16	0.07-0.14
7. Umbelliferone (Bhaskaran and Shruthi, 2016)	$C_9H_6O_3$	162.14	0.04-0.28
8. Rutin (Bhaskaran and Shruthi, 2016)	$C_{27}H_{30}O_{16}$	610.52	0.21-0.79
9. Ellagic acid (Bhaskaran and Shruthi, 2016)	$C_{14}H_6O_8$	302.197	0.23-5.92
10. Quercetin (Bhaskaran and Shruthi, 2016)	$C_{15}H_{10}O_7$	302.236	0.13-0.41
11. Kaempferol (Bhaskaran and Shruthi, 2016)	$C_{15}H_{10}O_6$	286.23	0.63-3.98
12. Coumaric acid (Bhaskaran and Shruthi, 2016)	$C_9H_8O_3$	164.0473	0-0.015
13. 1,2,4-trihydroxynapthalene-2-O-ß-D-glucopyranoside (Akintunde, et al., 2017)	$C_{16}H_{18}0_8$	338.31	Not reported
14. 2,3,4,6 tetrahydroxyacetophenone-2-O-ß-D-glucopyranoside (Goswami, et al., 2011)	$C_{14}H_{18}O_{10}$	346.29	Not reported
15. 1,2,4-trihydroxynaphthalene-1,4-di-O-ß-D-glucopyranoside (Goswami, et al., 2011)	$C_{22}H_{28}O_{13}$	500.45	Not reported
16. Luteolin-7-O-ß-D-glucopyranoside (Goswami, et al., 2011)	$C_{21}H_{20}O_{11}$	448.38	Not reported

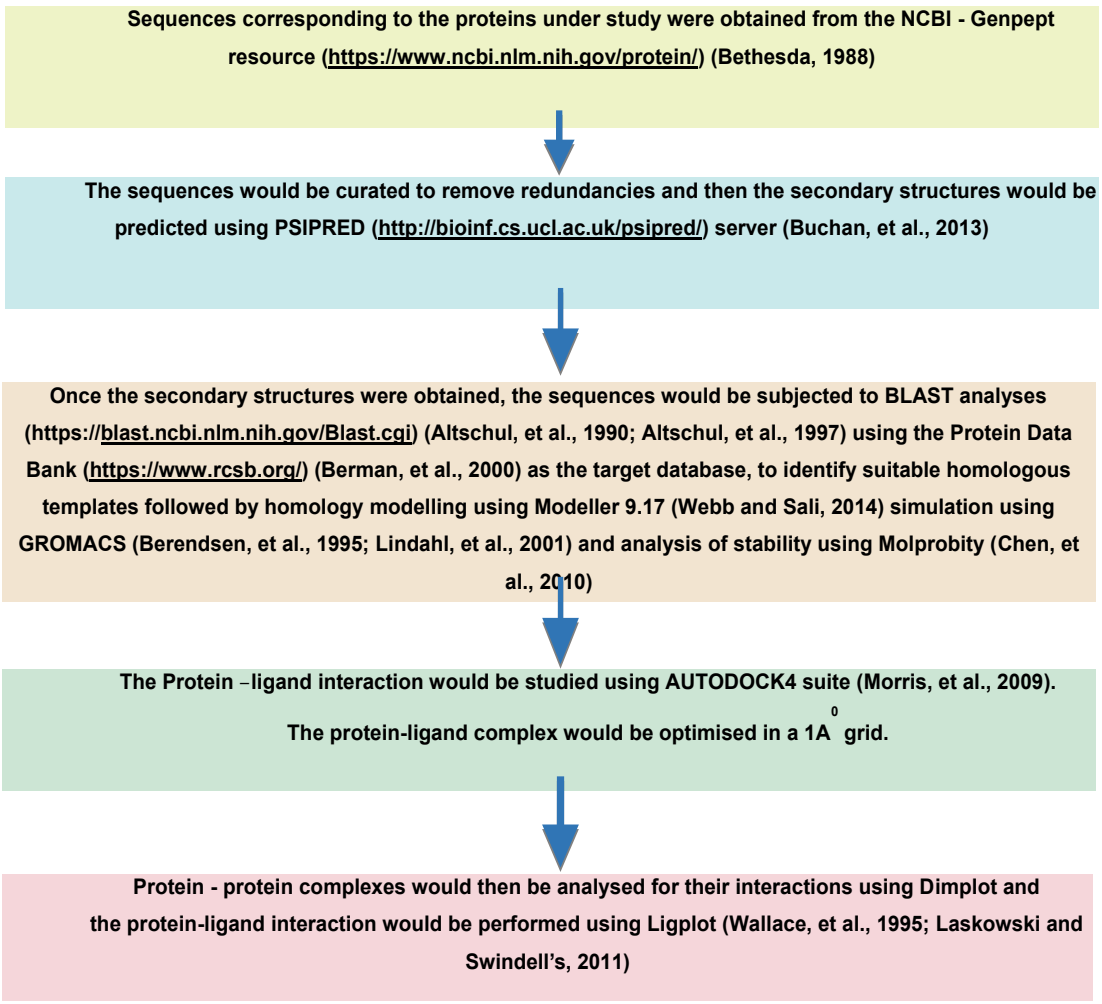

Figure 1. Potential steps towards Computer Aided Drug discovery using small molecule based bioactive compounds of *Lawsonia inermis* L

All the above active constituents should be incorporated in natural product libraries and passed through standard virtual screening pipelines (Fig. 1) and evaluated for their efficacies as potential lead compounds for therapeutic interventions.

CONCLUSION

Reports show that *Lawsonia inermis* L. is a plant, rich in phytochemicals which can be used to treat a vast range of human diseases like arthritis, diabetes, ulcers, inflammation, wound, blood sugar, microbial infection and many more. The potential action of the extract in inhibiting proliferation of cancer cells makes this plant seek all the more attention. Apart from the commercial and economic aspect, if the antioxidant and radical scavenging potential of the plant is exploited in a proper manner, then Henna plant can be potentially used to treat grave human diseases, like cancer. Also, *in silico* data analysis could be very helpful in the current times, for the development of potential drugs from the phytochemicals isolated. For this, focus should be on the correct manipulation of the parameters of extraction and the physiological conditions in which the plants grow. As the threat of microbial resistance and emerging infectious diseases gradually increase and mankind's therapeutic arsenal starts to dry up, more and more ethnopharmacologically important herbal explorations have to be undertaken to ascertain the continuous supply of lead molecules which can be formulated as drugs.

ACKNOWLEDGEMENT

This work was financially supported by DST.

REFERENCES

Abdillah S, Budiady I and Winarno H (2008). Hypoglycemic and antihyperlipidemic effects of henna leaves extract (*Lawsonia inermis* Linn) on Alloxan induced diabetic mice. Jordan J. of Pharma. Sc. 1(2): 126-131.

Akintunde O, Oyetunji OO, Eunice AO (2017). Studies on antibacterial potential and time kill assays of methanolic leaf extracts of *Lawsonia inermis* Linn. Asian J. of Res. in med. and Pharmaceutical Sc. 28;1(2): 1-9. DOI: 10.9734/AJRIMPS/2017/35195

Al-Damegh MA (2014). Evaluation of antioxidant activity effect of Henna (*Lawsonia inermis*) leaves and or vitamin C in rats. Life Sci J. 11(3): 234-241.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). "Basic local alignment search tool." J. Mol. Biol. 215: 403-410. PubMed

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman, DJ (1997). "Gapped BLAST and PSI-BLAST: A new generation of protein database search programs." Nucleic Acids Res. 25: 3389-3402. PubMed

Arayne MS, Sultana N, Mirza AZ, Zuberi MH, Siddiqui MH (2007). *In vitro* hypoglycemic activity of methanolic extracts of some indigenous plants. Pak. J. Pharm. Sci. 20(4): 261-268.

Badhai S, Barik DP, Mallick BC (2016). *In vitro* assays to show the antioxidant potential of ß-sitosterol from *Lawsonia inermis* leaves. Int. J. of Sc. and Res. 5(2): 2127-2130. https://doi.org/10.21275/v5i2.nov161724

Basipogu B, Syed NB (2015). Gastro protective activity of *Lawsonia inermis* (henna), a well-known medicinal plant. Int. J. Appl. Res. 1(11): 833-837. http://dx.doi.org/10.22271/allresearch.

Berendsen HJC, van der Spoel D, van Drunen R (1995). In: Computer Physics Communications. 91: 43-56. doi.org/10.1016/0010-4655(95)00042-E

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000). The Protein Data Bank. Nucleic Acids Res. 28: 235-242. doi:10.1093/nar/28.1.235

Bhaskaran K, Shruthi B (2016). Hepatoprotective activity of ethanolic seed extract of *Lawsonia inermis* against paracetamol induced liver damage in rats. Sch. J. App. Med. Sci. 4(7C): 2488-2491. https://doi.org/10.21276/sjams.2016.4.7.38

Brown D, Superti-Furga G (2003). Rediscovering the sweet spot in drug discovery. Drug Discov. Today. 8: 1067–1077. doi: 10.1016/S1359-6446(03)02902-7

Buchan DWA, Minneci F, Nugent TCO, Bryson K, Jones DT (2013). Scalable web services for the PSIPRED Protein Analysis Workbench. Nucleic Acids Res. 41 (W1): W340-W348.

Chaibi R, Drine S, Ferchichi A (2017). Chemical study and biological activities of various extracts from *Lawsonia inermis* (Henna) seeds. Acta Medica Mediterranean. 33: 981-986. DOI: 10.19193/0393-6384_2017_6_155

Chen VB, Arendall WB, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC (2010). MolProbity: all-atom structure validation for macromolecular crystallography. ActaCrystallogr D BiolCrystallogr. 66: 12-21. doi: 10.1107/S0907444909042073

Cheng T, Li Q, Zhou Z, Wang Y, Bryant SH (2012). Structure-based virtual screening for drug discovery: a problem centric review. AAPS J. 14: 133–141. doi: 10.1208/s12248-012-9322-0

Dhouafli Z, Jannet HB, Mahjoub B, Leri M, Guillard J, Tounsi MS, Stefani M, Hayouni EA (2017). 1,2,4-trihydroxynaphthalene-2-O-ß-D-glucopyranoside: A new powerful antioxidant and inhibitor of $Aß_{42}$ aggregation isolated from the leaves of *Lawsonia inermis.* Nat. Prod. Res. 29;33(10): 1406-1414. https://doi.org/10.1080/14786419.2017.1419229

Ehret T, Torelli F, Klotz C, Pedersen AB, Seeber F (2017). Translational rodent models for research on parasitic protozoa–a review of confounders and possibilities. Front. Cell. Infect. Microbiol. 7: 238. doi: 10.3389/fcimb.2017.00238

Eldrini S, Rahmat A, Ismail P, Taufiq-Yap YH (2007). Comparing of cytotoxicity properties and mechanism of *Lawsonia inermis* and *Strobilanthes crispus* extract against several cancer cell lines. J. Med. Sci. 7(7): 1098-1102. https://scialert.net/abstract/?doi=jms.2007.1098.1102

FitzGerald GA (2008). Drugs, industry, and academia. Sci. 320: 1563. doi: 10.1126/science.1161006

Goswami M, Kushreshtha M, Rao CV, Yadav S, Yadav S (2011). Anti-ulcer potential of *Lawsonia inermis* L. leaves against gastric ulcers in rats. J. of App. Pharmaceutical Sci. 1(2): 69-72.

Habbal O, Hasson SS, El-Hag AH, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, MS Al-Balushi, Al-Jabri AA (2011). Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. Asian Pac. J. Trop. Biomed. 1(3): 173-176. Doi:10.1016/S2221-1691(11)60021-X.

Hsouna AB, Trigui M, Culioli G, Blache Y, Jaoua S (2010). Antioxidant constituents from *Lawsonia inermis* leaves: isolation, structure elucidation and antioxidative capacity. Food Chem. 125(1): 193-200. https://doi.org/10.1016/j.foodchem.2010.08.060

Hunter P (2008). The paradox of model organisms: the use of model organisms in research will continue despite their shortcomings. EMBO Rep. 9: 717–720. doi: 10.1038/embor.2008.142

Hussain T, Arshad M, Khan S, Sattar H, Qureshi MS (2011). In vitro screening of methanol plant extracts for their antibacterial activity. Pak. J. Bot. 43(1): 531-538.

Kadhem M (2016). Anti-Arthritic Activity of Ethanolic Extract of *Lawsonia inermis* in Freund'S Adjuvant Induced Arthritic Rats. J. of Agric. and Vet. Sci. 9(6) Ver. II: 01-06.

Kumar KS, Kathireswari P (2016). Biological synthesis of silver nanoparticles (Ag-NPS) by *Lawsonia inermis* (Henna) plant aqueous extract and its antimicrobial activity against human pathogens.Int.J.Curr.Microbiol.App. Sci. 5(3): 926-937. http://dx.doi.org/10.20546/ijcmas.2016.503.107

Kumar M, Kumar S, Kaur S (2014). Identification of polyphenols in leaf extracts of *Lawsonia inermis* L. with antioxidant, antigenotoxic and antiproliferative potential. Int. J. of Green Pharm. 8(1): 23-36.https://www.researchgate.net/deref/http%3A%2F%2Fdx.doi.org%2F10.4103%2F0973-8258.126816

Laskowski RA, Swindells MB (2011). Ligplot+: Multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model. 51: 2778-2786.

Lila MA, Raskin I (2005). Health-related interactions of phytochemicals. J. Food Sci. 70: R20–7.

Lindahl E, Hess B, van der Spoel, D (2001). GROMACS 3.0: A package for molecular simulation and trajectory analysis. J. Mol. Model. 7: 306-317. doi.org/10.1007/s008940100045

Mansoor S, Mehfooz S, Mustafa H (2016). Antibacterial, antioxidant and cytotoxic potential of aqueous and organic extracts of *Lawsonia inermis*. World J. of Pharmaceutical Research. 5(5): 686-703. DOI: 10.20959/wjpr20165-5965.

Mohamed MA, Eldin IMT, Mohammed AH, Hassan HM (2016). Effects of *Lawsonia inermis* L. (Henna) leaves' methanolic extract on carbon tetrachloride induced hepatotoxicity in rats. J. Intercult Ethanopharmacol. 5(1): 22-26. https://dx.doi.org/10.5455%2Fjice.20151123043218

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 30: 2785-2791. doi: 10.1002/jcc.21256

Munos B (2009). Lessons from 60 years of pharmaceutical innovation. Nat. Rev. Drug Discover. 8: 959. doi: 10.1038/nrd2961

Naseem T, Farukh MA (2015). Antibacterial activity of green synthesis of iron nanoparticles using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract. J. of Chem. 21: 1-7. http://dx.doi.org/10.1155/2015/912342

National Center for Biotechnology Information (NCBI) [Internet]. Bethesda (MD): National Library of Medicine (US), Nat. Center for Biotech. Inform.; 1988;

Nesa L, Munira S, Mollika S, Islam Md. M, Choin H, Choudhury AU, Naher N (2014). Evaluation of analgesic, anti-inflammatory and CNS depressant activities of methanolic extracts of *Lawsonia inermis* barks in mice. Avicenna J. Phytomed. 4(4): 287-296. https://www.ncbi.nlm.nih.gov/pubmed/25068143

Ojewunmi OO, Oshodi T, Ogundele OI, Micah C, Adenekan S (2013). *In vitro* antioxidant, antihyperglycemic and antihyperlipidemic activities of ethanol extracts of *Lawsonia inermis* leaves. Brit. J. of Pharma. Res. 25;5(3): 301-314

Omran R, Al-Taee ZM, Hashim HO, Al- Jassani MJ (2017). Extraction of phenolic compounds as antioxidants from some plants and their cytotoxic activity against breast cancer cell line. Asian J. Pharm. Clin. Res. 10(7): 349-355. http://dx.doi.org/10.22159/ajpcr.2017.v10i7.18673

Phirke SS and Saha M (2013). *Lawsonia inermis* L.: A rainfed ratoon crop. National conference on Biodiversity: Statuses and Challenges in Conservation – FAVEO. 2013: 189-193.

Rahiman FA, Mahammad N, Taha RM, Elias H, Zaman FH (2013). Antimicrobial properties of *Lawsonia inermis* syn *Lawsonia alba*, *in vivo* and *in vitro*. Journal of Food, Agric. And Env. 11(3&4): 502-504. https://doi.org/10.1234/4.2013.4692

Rahmoun NM, Boucherit-Otmani Z, Benabdallah M, Villemin D, Choukchou-Braham N (2012). Antifungal and antibacterial activity of lawsone and novel naphthoquinone derivatives. Med. Et. Malad. infectious. 7;42(6): 270-275. https://doi.org/10.1016/j.medmal.2012.05.002

Raja W, Ovais M, Dubey A (2013). Phytochemical screening and antibacterial activity of *Lawsonia inermis* leaf extract. Intl. J. Microbiol. Res. 4(1): 33-36. DOI: 10.5829/idosi.ijmr.2013.4.1.6679

Ramsay RR, Popovic-Nikolic MR, Nikolic K, Uliassi E, Bolognesi ML (2018). A perspective on multi-target drug discovery and design for complex diseases. Clin. Transl. Med. 7: 3. doi: 10.1186/s40169-017-0181-2

Ramya A, Vijayakumar N, Renuka M (2015). Antiarthritic effect of aqueous extract of *Lawsonia inermis* L. An *in vitro* study. Int. J. Modn. Res. Revs. 3(8): 744-747.

Rizvi ZF, Mukhtar R, Chaudhary MF, Zia M (2013). Antibacterial and antifungal activities of *Lawsonia inermis*, *Lantana camara* and *Swertia angustifolia*. Pak. J. Bot. 11;45(1): 275-278.

Saeed SMG, Sayeed SA, Ashraf S, Naz S, Siddiqi R, Ali R, Mesaik MA (2013). A new method for the isolation and purification of lawsone from *Lawsonia inermis* and its ROS inhibitory activity. Pak. J. Bot. 45(4): 1431-1436. https://www.researchgate.net/publication/245542650

Sharma KK, Saikia R, Kotoky J, Kalita JC, Devi R (2011). Antifungal activity of *Solanum melongena* L., *Lawsonia inermis* L., *Justicia gendarusa* B., against dermatophytes. Int. J. PharmaTech. Res. 3(3): 1635-1640. http://www.sphinxsai.com/Vol.3No.3/pharm/pdf/PT=64(1635-1640)JS11.pdf

Singh DK, Luqman S (2014). *Lawsonia inermis* (L.) A perspective on anticancer potential of Mehndi/ Henna. Biomed. Res. and Therapy. DOI: 10.7603/s40730-014-0018-1

Singh P, Jain K, Jain B, Khare S (2012). *In vitro* micropropagation of *Lawsonia inermis*: an important medicinal plant. Int.Jr.C.R.R. 4(22): 29-34.

Sravanthi P, Kumar KM, Begum DT, Kumar SN, Ravindra reddy K, Khanhare RS (2011). Antiulcer activity of ethanolic extracts of *Lawsonia inermis* against cold stress induced ulceration in wistar rats. Journal of Pharmacy research. 4(6): 1872-1874.

Suleiman EA, Mohamad EA (2014). *In vitro* activity of *Lawsonia inermis* (Henna) on some pathogenic fungi. Journal of Mycology. 23;2014: 1-5. http://dx.doi.org/10.1155/2014/375932

Uma DB, Ho CW, Wan Aida WM (2010). Optimization of extraction parameters of total phenolic compounds of henna (*Lawsonia inermis*) leaves. Sans Malaysian. 39(1): 119-128.

Uthayakumar V, Chandrasekar R, Sreedevi PR, Senthil Kumar D, Jayakumar R, Balasubramanian V (2014). Immunostimulatory effect and disease resistance induced by *Lawsonia inermis* against *Aphanomyces invadans* in stripped murrels (*Channa striatus*). Malaya J. of Biosci. 1(4): 231-241.

Veeresham C (2012). Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res. 3(4): 200-1. doi: 10.4103/2231-4040.104709. PMID: 23378939; PMCID: PMC3560124

Wallace AC, Laskowski RA, Thornton JM (1995). LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. 8: 127-134. DOI:10.1093/protein/8.2.127

Webb B, Sali A (2014). Comparative Protein Structure Modeling Using Modeler. Current Protocols in Bioinformatics, John Wiley & Sons, Inc. 5.6.1-5.6.32.

Welsch ME, Snyder SA, Stock well BR (2010). Privileged scaffolds for library design and drug discovery. Curr. Opin. Chem. Biol. 14: 347–361. doi: 10.1016/j.cbpa.2010.02.018

Widyawati T, YS Pane, NA Yusuf (2019). Effect of *Lawsonia inermis* Linn. Extracts on Blood Glucose Level in Normal and Streptozotocin-Induced Diabetic rats. T. Pak. J. Nutr. 18(7): 671-676. DOI: 10.3923/pjn.2019.671.676

Yigit D (2017). Antifungal activity of *Lawsonia inermis* (Henna) against clinical *Candida* isolates. Journal of Science and Technology. 10(2): 196-202. https://doi.org/10.18185/erzifbed.328754

Yusuf M (2016). Phytochemical analysis and antibacterial studies of *Lawsonia inermis* leaves extract. J. Chem. Pharm. Res. 8(3): 571-575.

Zohourian TH, Quitain AT, Sasaki M (2011). Polyphenolic contents and antioxidant activities of *Lawsonia inermis* leaf extracts obtained by microwave assisted hydrothermal method. J. of Micro.

Powerand Electromagnet. Energy. 9;45(4): 193-204. https://doi.org/10.1080/08327823.2011.11689814

Zubardiah L, Mustaqimah DN, Auerkari EI (2012). Effectiveness of *Lawsonia inermis* Linnaeus leaves infusion in gingivitis healing. dentika Dental Journal. 17(2): 105-110