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Original Research Article

Behavioral, Analgesic, Anti-Inflammatory and Acute Oral Toxicity Studies on Poly-Herbal Formulation with Anti-Oxidant and Anti-Cancer Effects

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ABSTRACT

The study was aimed at carrying out pre-clinical studies on the poly-herbal formulation having evidence-based anti-oxidant and anti-cancer properties. The study involved the following pre-clinical studies on the poly herbal drug: behavioral studies were carried out at doses 50 mg and 100 mg/kg respectively using open field and light and dark methods; analgesic activity based on acetic acid induced writhing test was performed at 50 mg and 100 mg/kg doses of the extract; anti-inflammatory activity of the poly-herbal extract was conducted at the following doses: 10, 50, 100, and 150 mg/ml. Acute oral toxicity studies on the herbal extract were carried out at 5, 50, 300, 2000 and 5000 mg/kg doses. The results of pre-clinical studies carried out were comparable to the standards used endorsing the efficacy and safety of the herbal formulation under test. The efficacy and safety results of the activities carried out on the poly-herbal anti-oxidant and anti-cancer formulation by the researchers under test in this study and that of the already reported pharmacognostic, chemical, cytotoxic, anti-microbial and anti-oxidant results are suggestive of initiation of clinical studies on this supportive anti-oxidant and anti-cancer formulation.

***Keywords*:** Analgesic activity, Anti-inflammatory, Light and dark test, Open field test, toxicity.

Introduction

The current era is a period of advancements in formulation of medicines of natural origin, especially of herbal origin. Poly-herbal medicines are formulated nowadays for the supportive treatment of different diseases due to the synergistic efficacious effect of the herbals combined to make a formulation with no herb-conventional drug, herb-herb and herb-food interactions. [1] The herbals incorporated in the formulation contain polyphenols, flavonoids and tannins that exhibits analgesic, anti-microbial, anti-oxidant and anti-inflammatory effects. The World Health Organization (WHO) facilitates the use of natural origin medicines. Around the world especially in developing countries herbal remedies are used by more than 80% of the population. [2-4]
The composition of herbal formulation includes *Curcuma longa*, *Nigella sativa*, *Allium sativum*, *Zingiber officinale*, *Cinnamomum zeylanicum* in a specific ratio. According to the research work carried out on the poly-herbal formulation under current study has significant anti-oxidant and anti-cancer effects. [5]

Materials and Methods

Experimental animals
Mice strain nmri and rats wistar *srain* were obtained from Animal House, Dow University of Health Sciences - Karachi, Pakistan. They were used to carry out behavioral studies, analgesic activity, anti-inflammatory activity and acute oral toxicity studies. Animals were kept in cages with access to food and water.

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They were maintained in a light controlled room 30°C + 1°C 12/12 hours light/dark cycle) at least 7 days before administration of the drug. Animals used for neuro-pharmacological activity were acclimatized first for at least 5 days in the laboratory environment with 12 hours light and 12 hours dark schedule. Animals were housed in standard metal cages and provided food and water ad-libitum. Ethical Principles and Guidelines for Experiments on Animals formulated jointly by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences were followed to carry out animal studies.

Plant material and extraction
Herbal ingredients; *Zingiber officinalis* (rhizome), *Curcuma longa* (root), *Nigella sativa* (seeds), *Allium sativum* (bulb), and *Cinnamon zeylanicum* (barks) were obtained from Karachi, Pakistan in January 2022. All the plants were authenticated by Dr Farah Saeed and assigned voucher number (ACCNZ-01-22). The different parts of all the five plants were chopped into small pieces, shade dried at ambient temperature, and stored in air-tight container. It was ground into coarse powder in a grinder whenever required. After drugs size was reduced it was passed through sieve of mesh size 18 to obtain desire particle size of our drug. Obtained sieved mass of drug was weighted individually and mixed together in specific ratio (*Curcuma longa: Nigella sativa: Allium sativum: Zingiber officinalis: Cinnamon zeylanicum* - 2: 1: 1: 2: 1) in the container by simple hand mixing. Soxhlet's apparatus was used to prepare the extract. Ethanol was used as a solvent for extraction. Then extract was air dried in the air.

Behavioral studies
Assessment of neuro-pharmacological activity was studied by using open field test, and light and dark test. The tests were performed according to the protocol described by Irwin (1964). [6]
In each test, animals were divided into 4 groups (Control, Test 1 – 50 mg, Test 2 - 100 mg and Standard). Each group comprised of 5 animals. Lorazepam 0.5 mg/kg orally was used as standard. The crude drug and the Lorazepam were dissolved in distilled water and administered orally. The control animals were treated orally with same volume of saline as the crude extract. In all the tests, observations were made after 30 to 40 minutes of oral dose of the test substance.

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Open Field Activity
The open field apparatus designed in the laboratory was as described by Irwin (1964) i.e. consists of 76 x 76 cm square area with opaque walls 42 cm high. The floor is divided by lines into 25 equal squares. 25 to 30 gm weight mice were used in this experiment. Test was performed as described by Kennett *et al.*, (1985) and Turner (1965).[6-8]
Animals taken out from the cages and were placed in the center square of the open field (one at a time). Number of squares crossed with all four paws was counted for 30 minutes. Activities of control mice and drug treated mice were monitored in a balanced design to avoid order effect.

Light dark test
Light and dark test is one of the apparatus designed to observe anxiolytic behavior in mice. The apparatus consists of a plastic box with two compartments one of which is made of transparent plastic and the other of black colour plastic. Each animal is placed at the center of the transparent compartment and then the number of entries in each space, as well as, time spent in each compartment is recorded for 30 min.

Analgesic activity (Writhing test)
Male nmri strain mice (20–25 gm) were used in this experiment. 30 min. after the administration of poly-herbal extract (50 and 100 mg/kg i.p respectively), mice were administered an i.p. injection of 0.7 % v/v acetic acid solution. The mice were placed individually in transparent cages and 5 min were allowed to elapse. As per standard protocol acetic acid induced writhes were counted for a period of 20 minutes. For the purpose of scoring, a writhe was indicated by stretching of the abdomen and/or simultaneous stretching of at least one hind limb. Control animals were injected normal saline (10 ml/kg, i.p.), and the standard drug - Aspirin (10 mg/kg, i.p.).[9]

Anti-inflammatory Bioassay (in vitro)
Inhibition of Protein Denaturation Assay: The reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract, by which the concentrations (mg/ml) became 10, 50, 100, and 150. Disprin was used as standard drug. The mixtures were incubated at 37°C ± 2°C in a biological oxygen demand incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was determined by spectrophotometer at 660 nm using vehicle as blank. Test extracts were chosen such that, they remained the nearest possible to the standard therapeutic mode. The percentage inhibition of protein denaturation was calculated by using the following formula: [10-12]

$$\% \text{ inhibition} = 100 \times ([Vt/Vc] - 1).$$

Where, Vt = absorbance of test sample, Vc = absorbance of control

Acute oral toxicity test
Healthy female wistar rats were used for the study as per the standard criteria of LD_{50} test. Poly herbal extract was administered at different dose levels 5, 50, 300, 2000 and 5000 mg/kg. Animals were observed for the following signs of toxicity: changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somato-motor activity and behavior pattern. The animals were vigilantly observed for the signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. [13]

Statistical analysis
Results of the study were presented as mean plus minus standard error of mean (M±SEM). Differences between control and treatment groups were analyzed by student-t test. [14]

Results and Discussion

Behavioral Studies
Open field activity
The groups given poly herbal extract were found to be active when compared to the standard and control groups [Figure 1].

Light and dark box activity
Results of the light and dark box activity revealed that the groups administered poly-herbal extract were active and spent more time in light box as compared to the standard and control group [Figure 2].

Analgesic Activity (acetic acid induced writhing test)
Our study results exhibited pronounced analgesic effect of the poly-herbal extract at the dose of 100 mg comparable to the standard drug, Aspirin [Figure 3].

Anti-inflammatory activity (Inhibition of Protein Denaturation Assay)
At higher dose of 150 mg the poly herbal extract displayed prominent anti-inflammatory activity comparable to the standard drug [Figure 4].

Acute Oral Toxicity Test
The acute oral toxicity test was performed in experimental laboratory animals (rats) at different dose levels 5, 50, 300, 2000 and 5000 mg/kg. Throughout the study no mortality was found and no toxic signs such as changes in skin, fur, eyes and mucous membrane, behavior pattern, tremors, salivation, diarrhea and coma were observed. The results of the study exhibited pronounced analgesic, anti-inflammatory effects. No toxic effects were observed. Behavioral studies revealed that the test groups administered poly-herbal extract were more active. The treated group exhibited the distinct analgesic and anti-inflammatory activity. The poly herbal extract was found to be non-toxic. The results justified the safety and efficacy of the poly-herbal extract. The composition of the poly herbal extract contains the ingredients that have been individually tested and reported to be safe and effective. The study is in continuation of the standardization work on this poly herbal drug by Farah-Saeed *et al.* 2021.[5]

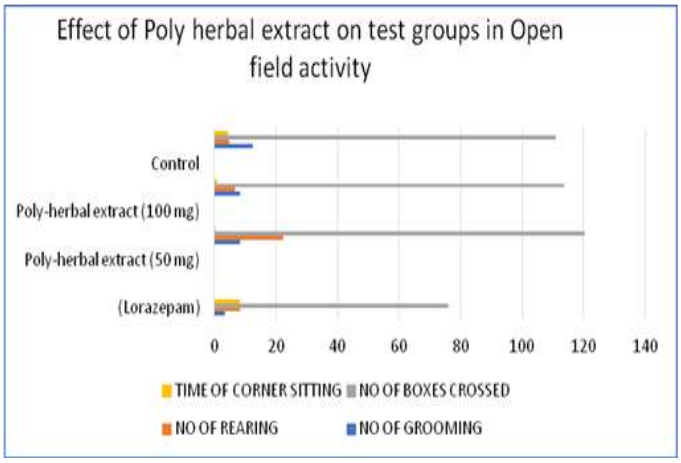

Figure 1: Poly-herbal extract effect on mice behavior using open filed apparatus

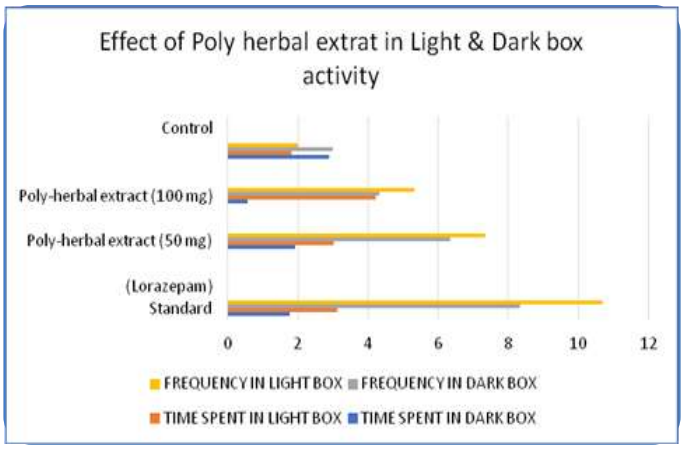

Figure 2: Poly-herbal extract effect on mice behavior using light and dark box apparatus

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The positive efficacious and safety results of our formulation are comparable to the results of the research work carried out on each of the ingredient separately by various scientist that are included in the formulation are mentioned below. All the ingredients present in the formulation have documented evidence based data of proved efficacy and safety on the basis of their active constituents. Patiño-Morales *et al*. 2021 have mentioned the use of *Allium sativum* in treatment of numerous pathologies including cancer. [15] The active constituents of garlic includes organosulfur compounds: alliin, allicin, S-allylcysteine, diallyl sulfide, diallyl disulfide, and diallyl trisulfide; flavonoids; phenolic compounds; vitamins, enzymes, minerals (iron, manganese, magnesium, calcium, selenium) and amino acids. These constituents may potentiate anti-oxidant, anti-microbial, analgesic, anti-inflammatory and anti-cancer effects. [16-21]

Curcuma longa contains carbohydrates, alkaloids, phenols, flavonoids, terpenoids, tannins, steroids and glycosides. Curcumin, a hydrophobic polyphenol compound is major active constituent of *Curcuma longa*.

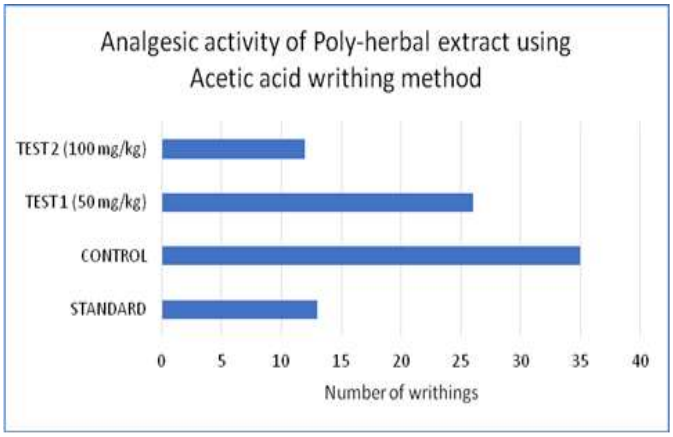

Figure 3: Analgesic effect of poly-herbal extract using acetic acid writhing

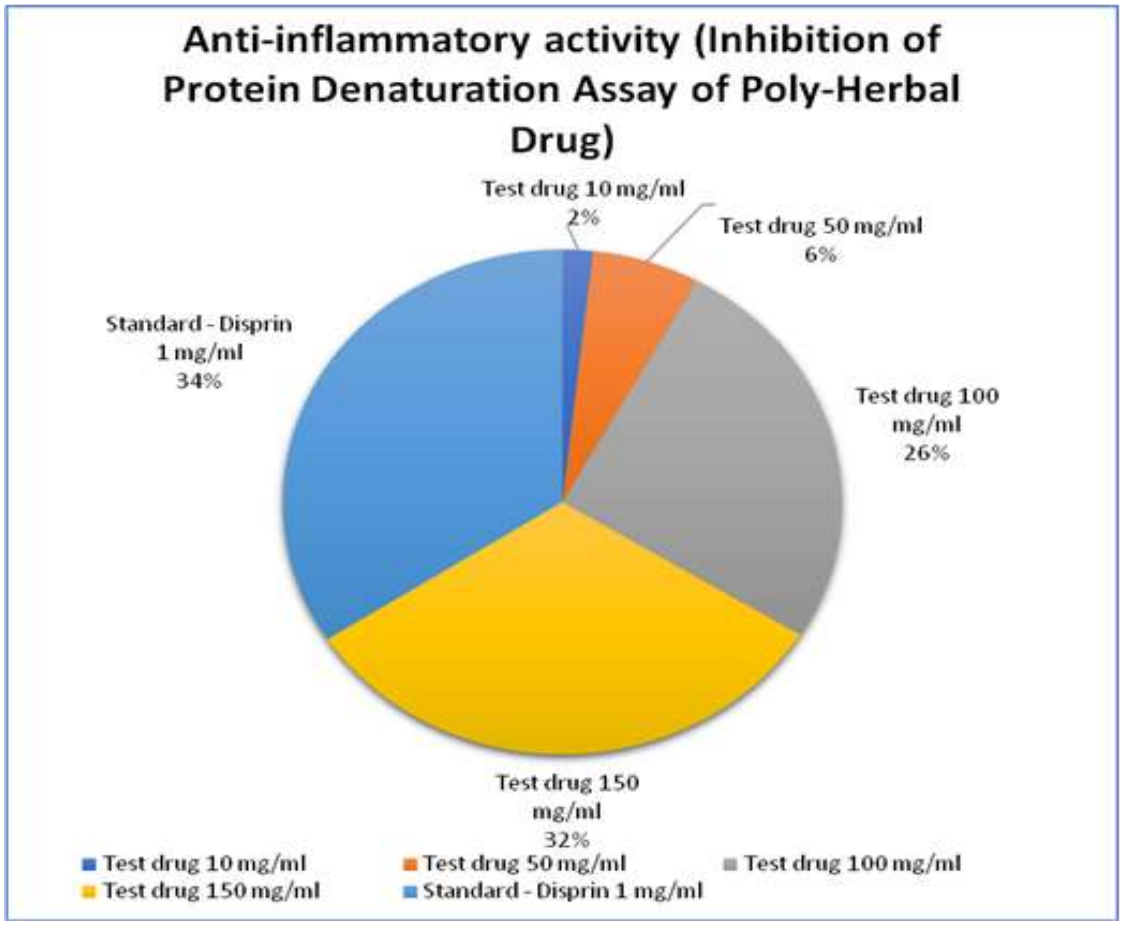

Figure 4: Anti-inflammatory activity (Inhibition of Protein Denaturation Assay) of Poly-Herbal Extract

It exhibits anti-oxidant, anti-microbial, analgesic, anti-inflammatory and anti-cancer effects. [22-24] *Cinnamon zeylanicum* primarily contains flavonoids, volatile oils including borneol, eugenol and cinnamaldehyde that are the most explored due to their active pharmacological effect. It has anti-microbial, anti-inflammatory, anti-oxidant and anti-cancer effects. [25-28] *Nigella sativa* contains volatile oil, phenolic acids, flavonoids, fixed oil, proteins, amino acids, reducing sugars, alkaloids, tannins, resins, glycosides, vitamins and minerals. Amongst the volatile oils, thymoquinone is the major compound present in it. Nigella sativa possess pronounced pharmacological activities including anti-oxidant, immunomodulating, anti-cancer, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory and anti-microbial. [29-32] *Zingiber officinale* contains volatile oils, steroids, diarylheptanoids, phenyl *alkanoids*, monoterpenoid glycosides, carbohydrates, proteins and sulfonates. *Zingiber officinale* has analgesic, anti-inflammatory, anti-microbial, anti-oxidant, anti-diabetic, anti-emetic, anti-cancer, radio-protective effect. Anti-cancer effect of ginger is majorly attributed to gingerols and shogaols present in it. [33-36]

Conclusion

The results revealed that the poly herbal formulation possesses analgesic and anti-inflammatory activities and is relatively safe as endorsed by oral acute toxicity study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, Dubey SP, Khan N, Jain DK. Poly-herbal Formulation Concept for Synergic Action: A Review. J Drug Deliv and Therapeut. 2019; 9(1-s):453-466.
2. Sane RT. Standardization, quality control and GMP for herbal drug. Indian drugs 2002; 39(3):184-190.
3. Farnsworth NR, Akerele O, Bingle AS, Sojarto DD, Guo Z. Medicinal plant in therapy. Bulletin of the World Health organization. 1985; 63:965-981.
4. Abraham R and Paridhavi M. A review of comprehensive study on medicinal plants of poly-herbal formulation- Churna. Asian J Pharm Clin Res. 2013; 6(4):11-18.
5. Farah-Saeed, Akhter H, Raza U, Ali MW, Nasir H. Preparation and Standardization of an Anti-Oxidant Poly-Herbal Formulation: Prospective Anti-Cancer Medicine. J Compl and Alternat Med Res. 2021; 16(4):263-274.
6. Irwin S. 1964. Drug screening and evaluation of new compounds in animals; In Animal and Clinical Pharmacology Techniques in Drug Evaluation (Nodine JH and Seigler PE eds.) pp. 55 - 68. Yearbook Medical Publishers, Inc, Chicago. J Pesticide Sci. 2006; 31(1):29-34.
7. Kennett GA, Dickinson SL, Curzon G. Enhancement of some 5-HT depressant behavioral responses following repeated immobilization in rats. Brain Res. 1985; 330:253-263.
8. Turner RA. Anticonvulsant In: Screening Methods in Pharmacology, Academic Press, New York and London; 1965; 64-69.
9. Bukhari IA, Gilani AH, Meo SA, Saeed A. Analgesic, anti-inflammatory and antiplatelet activities of *Buddleja crispa*. BMC Comple and Alt Med. 2016; 1-7.
10. Dey P, Chatterjee P, Chandra S, Bhattacharya S. Comparative in vitro evaluation of anti-inflammatory effects of aerial parts and roots from *Mikania scandens*. J Adv Pharm Educ Res. 2011; 1:271-7.
11. Banerjee S, Chanda A, Adhikari A, Das AK, Biswas S. Evaluation of phytochemical screening and anti-inflammatory activity of leaves and stem of *Mikania scandens* (l.) wild. Ann Med Health Sci Res. 2014; 4:532-6.
12. Uwaya, Do, Okakwu R, Omozuwa, OP. *In-Vivo* and *In-Vitro* Anti-Inflammatory Activities of the Aqueous Extract of Di-Herbal Formulation (*Euphorbia hirta* and *Lactuca virosa*). J. Appl. Sci. Environ. Manage 2020; 24(11):1979-1985.
13. OECD (2002), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071001-en.
14. Snedecor GW and Cochrane WG. Statistical Methods. Sixth edition. Ames, Iowa: The Iowa State University press. 1967.
15. Patiño-Morales CC, Jaime-Cruz R, Sánchez-Gómez C, Corona JC, Hernández-Cruz EY, Kalinova-Jelezova I, Pedraza-Chaverri J, Maldonado PD, Silva-Islas CA, Salazar-García, M. Anti-tumor Effects of Natural Compounds Derived from *Allium sativum* on Neuroblastoma: An Overview. Antioxidants 2021; 11:48.
16. Gebhardt, R, Beck H, Wagner KG. Inhibition of cholesterol biosynthesis by allicin and ajoene in rat hepatocytes and HepG2 cels. Biochi. Biophys. Acta (BBA) Lipids Lipid Metab. 1994; 1213:57–62.
17. Eilat S, Oestraicher Y, Rabinkov A, Ohad D, Mirelman D, Battler A, Eldar M, Vered Z. Alteration of lipid profile in hyperlipidemic rabbits by allicin, an active constituent of garlic. Coron Arter Dis. 1995; 6:985–990.
18. Shadkchan Y, Shemesh E, Mirelman D, Miron T, Rabinkov A, Wilchek M, Osherov N. Efficacy of allicin, the reactive molecule of garlic, in inhibiting Aspergillus spp. in vitro, and in a murine model of disseminated aspergillosis. J Antimicrob Chemother. 2004; 53:832–836.
19. Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, Darley-Usmar VM, Doeller JE, Kraus DW. Hydrogen sulfide mediates the vasoactivity of garlic. Proc Natl Acad Sci USA. 2007; 104:17977–17982.
20. Zoccali C, Catalano C, Rastelli S. Blood pressure control: Hydrogen sulfide, a new gasotransmitter, takes stage. Nephrol. Dial. Transplant. 2009; 24:1394-1396.
21. Ozma MA, Abbasi A, Rezaee MA, Hosseini H, Hosseinzadeh N, Sabahi S, Noori SMA, Sepordeh S, Khodadadi E, Lahouty M, Hossein HS. A Critical Review on the Nutritional and Medicinal Profiles of Garlic's (Allium sativum L.) Bioactive Compounds, Food Reviews International, 2022. 1-38. https://doi.org/10.1080/87559129.2022.2100417.
22. Jogdand S and Bhattacharjee J. Evaluation of analgesic activity of turmeric (*Curcuma longa* Linn.) in Wister rats. Int J Basic Clin Pharmacol. 2017; 6:568-71.
23. Eke-Okoro UJ, Raffa RB, Pergolizzi JV Jr, Breve F, Taylor R Jr. For the NEMA Research Group. Curcumin in turmeric: Basic and clinical evidence for a potential role in analgesia. J Clin Pharm Ther. 2018; 43:460– 466.
24. Ify OA, Raphael1 AG, Tochukwu1 OC, Amarachi OUS, Ikechukwu NA, Madukaihe MJ, Thoma Y, Innocent OC. 2021. The Antimicrobial, Anti Inflammatory and Analgesic Activities of the Rhizome Extract of *Curcuma longa* L. (Turmeric). J Adv in Biol & Biotech; 24(6):1-16.
25. Jyotirmayee B and Mahalik G. A review on selected pharmacological activities of *Curcuma longa* L., Int J Food Properties. 2022; 25(1):1377-1398,
26. Thakur S, Walia B, Chaudhary G. Dalchini (*Cinnamomum zeylanicum*): a versatile spice with significant therapeutic potential. Int J Pharm Drug Anal. 2021; 9(2):126-136.
27. Kallel I, Hadrich B, Gargouri B, Chaabane A, Lassoued S. 2019. Optimization of cinnamon (*Cinnamomum zeylanicum* Blume) essential oil extraction: evaluation of antioxidant and anti-proliferative effects. Evid Based Complement Alternat Med. 2019; 6498347.
28. Christiany C, Sudrajat SE, Ika Rahayu I. The Potency of *Cinnamomum zeylanicum* to Prevent Diseases: A Review. Eureka Herba Indonesia. 2021; 2(1):49-58.
29. Parihar AKS, Mayank KK, Sahu U, Karbhal1 KS, Inchulkar SR, Shah K, Chauhan NS. Quality control of Dalchini (*Cinnamomum zeylanicum*): a review. Advan, Trad Med. 2021. 1–10. https://doi.org/10.1007/s13596-021-00547-w.
30. Gilani AH, Jabeen Q, Khan MAU. A Review of Medicinal uses and Pharmacological activities of *Nigella sativa*. Pakistan J Bio Sci. 2004; 7(4):441–451.
31. Dalli M, Bekkouch O, Azizi SE, Azghar A, Gseyra N, Kim B. *Nigella sativa* L. Phytochemistry and Pharmacological Activities: A Review (2019–2021). Biomolecules 2022; 12-20.
32. Hannan MA, Rahman MA, Sohag AAM, Uddin MJ, Dash R, Sikder MH, Rahman MS, Timalsina B, Munni YA, Sarker PP. Black Cumin (*Nigella sativa* L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. Nutrients 2021; 13:1784.
33. Ansary J, Giampieri F, Forbes-Hernandez TY, Regolo L, Quinzi D, Gracia VS, Garcia VE, Tutusaus PK, Alvarez-Suarez JM, Battino M. 2021. Nutritional Value and Preventive Role of *Nigella sativa* L. and Its Main Component Thymoquinone in Cancer: An Evidenced-Based Review of Preclinical and Clinical Studies. Molecules. 2021. 26 (8): 1 - 27.

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34. Zhang M, Zhao R, Wang D. 2021. Ginger (*Zingiber officinale* Rosc.) and its bioactive components are potential resources for health beneficial agents. Phytother Res. 2021; 35:711–742.
35. Pagano E, Souto EB, Durazzo A. Ginger (*Zingiber officinale* Roscoe) as a nutraceutical: Focus on the metabolic, analgesic, and anti-inflammatory effects. Phytotherapy Research. 2021; 35:2403–2417.
36. Dissanayake KGC, Liyanage WA, Waliwita C, Liyanage RP. A Review on Medicinal Uses of *Zingiber officinale* (Ginger). Inter J Health Sci and Res. 2020; 10(6):142-148.
37. Dubey S and Kushwaha S. A review on pharmacological properties of *Zingiber officinale*. IP J Nutr Metab Health Sci; 2022; 5(1):11-17.