Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1511-1516 | ISSN:2251-6727

Standardization and Quality Control Profile of *Acacia Nilotica* Using Pharmacognostic Analysis

Swati A. Tekale[1], Vijay N. Gunjkar[2*]

[1]Department of Pharmacology, Nanded Pharmacy College, Nanded, 431605, India
[2*]Department of Pharmaceutics, Nanded Pharmacy College, Nanded, 431605, India.

***Corresponding Author:** Vijay N. Gunjkar

(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

Keywords:

Quality control, *Acacia nilotica*, Asthma, Standardization, Extraction

Abstract

Background: Herbal Asthma treatment has more benefits than existing pharmaceutical therapies with inevitable side effects. *Acacia are* well-known *herbal* formulations that act as a nutritional supplement and active phytoconstituents from the plant used to prevent oxidative stress involved pathophysiology of various diseases. As herbal products are made up entirely extraction processes, it can cause a variety of health problems; therefore, it is essential to lay out quality control guidelines, for standardization of *herbal* formulations. *Acacia nilotica (AN*) is one of such *plant based herbal* formulation with use in improving various diseases preventive functions. Materials and methods: Our study was aimed to standardize and the quality control profile of AN herbal formulation. First, the *AN* was prepared in-house and evaluated for various physicochemical attributes. The total polyphenol and flavonoid content was determined by colorimetric assay. Also, the formulation was investigated for heavy metal and pesticide content. Results: The total polyphenol content and flavonoid content was found to be 1.57±0.4 mgEGA/g and 2.45±0.08 mgEQ/g respectively. The presence of higher content of polyphenols in *AN* could be responsible for neuroprotection. Also, the absence of heavy metals and pesticide residues in the formulation suggest their safe consumption by oral ingestion.

1. Introduction:

Herbal remedies in general have more beneficial effects than existing pharmaceutical therapies for complex and multifactorial diseases like Alzheimer's disease (AD) with inevitable side effects. The quality of life of patients can also be improved because they can be taken as nutraceutical and even a minor increase in dose would not be deleterious. However, a proper administration route has to be selected so that they reach the site and show therapeutic action to deliver these polyherbal formulations. The secondary metabolites also known as phytochemical compounds contained in plants are majorly responsible for the pharmacological actions. Phytochemicals affect the function of different receptors in the brain for neurotransmitters, both exciting and inhibiting, and thus help in the maintenance and regulation of the chemical balance of the brain[1].*Acacia Nilotica* (AN) has long been used as a folk cure for asthma, but little is known about how AN could possibly modulate this disease. *Acacia nilotica* seeds were collected. The seeds were cracked, washed, the shell removed and the kernels were pulverized for oil extraction. The oil was extracted by solvent extraction. Exactly 100g of A. nilotica seed kernel powder was placed in the thimble which was inserted in the centre of the extractor. The extraction was conducted for two hours. The preweighed flask containing the oil was cooled in the desiccator and the resulting mixture containing the oil was heated at 70oC to recover solvent from the oil and the percentage of oil extracted was determined. Herbal formulation *Acacia nilotica* (AN) is a medicinal preparation are known to play a significant role. A review of the literature shows that standardization strategies for AN are still lacking[2].

The principle of preparation is to boil oil for a long time with the prescribed, depending on the formulation[3]. Since oil is made up entirely of fat, it can cause a variety of health problems, and also this being an admixture of different herbs, there are strong possibilities of adulteration therefore adequate quality control and strict standardization guidelines need to be mandated for every *herbal* medicine. The origin and quality of raw materials are critical in ensuring the quality and consistency of herbal preparations. Other important factors affecting the nature and therapeutic

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1511-1516 | ISSN:2251-6727

value of herbal medicine include the freshness of the plant used, temperature, sun, cold exposure, insect bites, and the portion of the plant collected. This explains why herbal medicine comes in such a wide range of formulations. So, right from the correct raw material procurement to the finished and herbal formulations, it must be subjected to strict quality control and standardization[4].As a result, this research aims to underlay analytical specifications of *AN* with the determination of total polyphenol and flavonoid content to investigate pesticides and heavy metal contaminants.

2. Materials and methods:

2.1 Plant materials:

All plants were collected from the village Amrabad, District Nanded, Maharashtra. The plant was collected and authenticated by a botanist at Yeshwant Mahavidyalay, Nanded.

2.2 Chemicals and Reagents:

All chemicals used were of analytical grade and purchased from Vinit chemist, Nanded, Merck, USA.

2.3 Methods:

2.3.1 Preparation of *Acacia nilotica:* *AN* was prepared as reported procedures. The seeds were cracked, washed, the shell removed and the kernels were pulverized for oil extraction. The oil was extracted by solvent extraction. Exactly 100g of A. nilotica seed kernel powder was placed in the thimble which was inserted in the centre of the extractor. The extraction was conducted for two hours. The preweighed flask containing the oil was cooled in the desiccator and the resulting mixture containing the oil was heated at 70oC to recover solvent from the oil. These fatty acids are amphipathic in nature, thus a water-soluble constituent binds with the hydrophilic end and oil-soluble constituents bind with the hydrophobic end. The continuous agitation and heating during preparation enhances the extraction of phytoconstituents. After evaporation of the water, it contains both oil and water-soluble ingredients[5]. In the present study, *AN* was monitored at each step of the manufacturing process.

2.4 Analytical specifications of *AN*

2.4.1. Description of *AN*: The appearance, color, and odor of *AN* was observed manually. The volume was measured upon melting by heating until it is molten. The pH of the *AN* formulation was measured using a pH meter.

2.4.2. Moisture content: The formulation was taken in an evaporating dish of 0.5 g and allowed to dry at 105°C for 5 hrs. Before weighing. Continued the drying and weighed every hour until the difference in weight between two subsequent measurements was no greater than 0.25%. When there was less than a 0.01 g difference between two successive weigh-ins after drying, cooled for 30 mins in a desiccator, and the weights were noted, and calculated the moisture content using the below formula: -

$$\text{Loss on Drying } \left(\%\frac{w}{w}\right) = \frac{(W2-W3)}{(W2-W1)} \times 100 \quad (1)$$

Where, W1- Weight of empty crucible,
W2 - Weight of crucible with sample before heating,
W3 - Weight of crucible after drying

2.4.3. Microbial content: This test was performed as per the *reported procedure*[3].

2.4.4. Mineral oil content: Added 1ml of the sample to 22ml of the alcoholic KOH solution in a conical flask. Used a condenser to boil the solution in a water bath, until it turns clear and there are no greasy drips on the flask's edges. Removed the flask from the water bath and carefully added 25ml of boiling distilled water along the side of the test tube after transferring the contents to a warm, wide-mouthed test tube. Throughout the addition, kept shaking the tube lightly from side to side. The depth of turbidity depends on the amount of mineral oil present and the presence of turbidity indicates the existence of mineral oil[6].

2.4.5. Refractive index: The refractive index varies considerably, the temperature was carefully calibrated and maintained about the temperature. The refractive index was measured at 25°C concerning the wavelength of the sodium D line (=589.3 nm) using the Abbe Refractometer[7] (Model: NAR-1T LIQUID).

2.4.6. Viscosity: The AN was filled in a U-tube viscometer for viscosity measurement such that the fluid level was within 0.2mm of the viscometer's filling mark when the capillary was vertical and the test liquid reached the required temperature. The sample was sucked or blown until the required mark was reached, and then it was weighed. The following equation was used to measure the kinetic viscosity in centistokes[8].

$$F = \mu A \times \frac{\mu}{y} \quad (2)$$

F= force,
μ= viscosity of the liquid,
A= Area of each plate,
μ/y= rate of shear deformation,

2.4.7. Determination of acid value: Accurately weighed 10g of *AN* was taken into a measuring cup and 50 ml of a mixture of equivalent quantities of alcohol and solvent ether. It was neutralized with the addition of 1 ml of phenolphthalein solution to a 250 ml flask.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1511-1516 | ISSN:2251-6727

The material was fully melted by gently heating it in a water bath. Titration was carried out with 0.1N potassium hydroxide, the flask was constantly shaken and pink color was obtained that lasted for 15 sec. The acid value for *AN* was calculated using the formula:-

$$\text{Acid value} = \frac{\text{a} \times 0.00561 \times 1000}{\text{W}} \quad (3)$$

a - volume (ml) of 0.1 N potassium hydroxide required
W -Weight (g) of the substance taken

2.4.8. Determination of saponification value: Potassium hydroxide in the amount of 35-40 g was correctly weighed and dissolved in 20 ml of water. It was left to stand overnight before being poured with clear liquor. 2 g of AN was accurately weighed in a 250 ml tarred flask, 25 ml of alcoholic potassium hydroxide solution was added, a reflux condenser was attached, and the flask was boiled on a water bath for 1 hour with the contents of the flask being constantly rotated, cooled, and 1 ml of phenolphthalein solution was added, and the excess of alkali was titrated with 0.5 N HCl[9].The saponification value for *AN* was calculated using the formula as follows: -

$$Saponification\ value = \frac{(b-a) \times 0.02805 \times 1.000}{W} \quad (4)$$

W-Weight (g) of the substance taken
a- blank (ml) taken omitting the substance
b - Volume (ml) required

2.4.9. Determination of Iodine Value: The accurately weighed *AN* was placed in a dry iodine flask and 10 ml of carbon tetrachloride was added and dissolved. 20 mL of iodine monochloride solution was added, and a stopper that had been moistened with potassium iodide solution was inserted. Allowed to stand in a dark position at a temperature of about 17ºC for 30 min. 15 ml of the solution of potassium iodide and 100 ml of water were added, shaken, and titrated with 0.1 N sodium thiosulphate, using a starch solution as an indicator[10]. The iodine value for AN was determined using the formula: -

$$Iodine\ value = \frac{(b-a) \times 0.01269 \times 100}{W} \quad (5)$$

a - blank (ml) taken without the substance being tested
b- Volume(ml) of 0.1 N sodium thiosulphate required
W- Weight (g) of *AN*

2.4.10. Determination of peroxide value:5 g of correctly measured substance was put in a 250 ml glass stoppered conical flask, and 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform was added and swirled until it was dissolved. A 0.5 ml saturated potassium iodide solution was applied and left to stand for 1 min, shaken occasionally. 30 ml of water was added and titrated gradually with continuous and vigorous shaking with 0.01M sodium thiosulphate until the yellow color disappeared. A 0.5 ml starch solution was added, and the titration was continued with vigorous shaking until the blue color had faded completely (a ml). The process was repeated to examine the substance (b ml)[11]. The peroxide value for *AN* was calculated using the formula:-

$$Peroxide\ value = \frac{10 \times (a-b)}{W} \quad (6)$$

W - Weight (g) of the substance
a - blank (ml) taken without the substance being tested
b - Volume (ml) of 0.01 M sodium thiosulphate required

2.4.11. Total polyphenolic content (TPC): The Folin–Ciocalteu colorimetric method was used to determine the TPC of AN. In a nutshell, the sample (1 ml) was combined with Folin–phenol Ciocalteu's reagent (1 ml), then sodium carbonate (7 %, 10 ml) was added, and the volume was made up to 25 ml with distilled water. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer after 90 min of incubation in the dark at room temperature (Jasco V-530International Co., Ltd., Tokyo, Japan)[12,13]. Gallic acid was used for the construction of the calibration curve and the TPC for the *AN* sample was expressed as mgGAE/g.

2.4.12. Total flavonoid content (TFC): The aluminum chloride colorimetric method was used to evaluate the TFC of AN. Distilled water (4 ml) and 0.3 ml sodium nitrite (5%) were added to the sample extract (1 ml). After 5-6 mins, aluminum chloride (0.3 ml, 10%) and sodium hydroxide (2 ml, 1 M) were added, respectively. The volume was diluted to 10 mL with distilled water. At 510 nm, the absorbance was measured using a UV-Vis spectrophotometer (Jasco V-530 International Co., Ltd., Tokyo, Japan)[14,15]. Quercetin was used for the construction of the calibration curve and the TFC for the *AN* sample was expressed as mgQE/g.

2.4.13. Pesticide content: Pesticide analysis was done using a 410 Proster Binary LC with 500 MS IT PDA detectors from Varian Inc. Water (8 ml) and acetonitrile (10 ml in 1 % acetic acid) was added to 2 g of sample. This was supplemented with 6 g of anhydrous magnesium sulfate and 1.5 g of anhydrous sodium sulfate. The mixture was held in a desiccator for cooling after being heated at 150°C for 5 min. It was then vortexed for 3 min and centrifuged for 5 minutes at 4000 rpm. In a 15 ml polypropylene centrifuge tube, 5 ml of supernatant was combined with 25 mg of primary secondary amine, shaken for 30 sec, and centrifuged for 5 min at 10,000 rpm. 2 mL of the supernatant was combined with 200 µL of 10%

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1511-1516 | ISSN:2251-6727

diethylene glycol. At 35°C, the solution was evaporated to dryness under nitrogen. This solution was reconstituted with 1 ml of 0.1 % acetic acid and 1 ml of methanol, filtered through a 0.2 µm membrane filter, and injected (5–20 µL) into an LC-MS/MS system[16].

2.4.14. Heavy metal content: The modified PerkinElmer Corporation (1982) approach was used to analyze heavy metals for arsenic (As), copper (Cu), and lead (Pb). The ash was obtained by heating 0.5 g of AN first at 100°C to reduce moisture content, then at 500°C to achieve a constant crucible weight. Agilent Technologies 7700 series Inductively Coupled Plasma/MS (ICP-MS) was used to test three different metals: Cu, As, and Pb. By dissolving their ash in nitric acid (5 ml, concentration), water (5 ml), and hydrogen peroxide (5 ml), solutions containing As, Cu and Pb ions were obtained (1 ml). In the ash solution, As, Cu, and Pb were measured directly. Dilutions of stocks were used to build calibration curves[17, 18].

3. Results:

3.1. Physicochemical parameters of *AN*

The organoleptic and physicochemical characteristics were found to be within the range of the description given in the *Ayurvedic Pharmacopeia of India*. The results are reported as the average and given in Table 2.

Table 2: The observed and standard values for the physicochemical properties of *Acasia nilotica*

Sr No.	Properties	Observed value	Standard value
1	Refractive index at 40^0 C	1.31	1.34- 1.35
2	Specific gravity &weight per mL at 40°C (g)	0.87& 0.83	0.88 & 0.84
3	pH	4.36	Below 6.1
4	Saponification value	157.57	166.67
5	Iodine value	32.48	33-35
6	Acid value	1.95	1.97
7	Peroxide value	5.42	NMT 6
8	Congealing point (°C)	20-17	22-17
9	Moisture content (%)	2.70	2.80
10	Mineral oil content	Absent	Absent
11	Microbial limits	Absent	Absent

3.2. Total polyphenol and flavonoid content:

The TPC of *AN*was estimated from the linear regression equation (y=0.148x-0.003), obtained from the standard calibration curve of gallic acid. The TPC of *AN* was found to be 1.57±0.4 mgEGA/g. The TFC of *AN* was found to be 2.45±0.08 mgEQ/g calculated from the linear regression equation (y=0.040x-0.072) derived from the standard calibration curve of quercetin.

3.3. Heavy metal content:

By measuring the peak area of the selected analytes concerning the internal standard, quantification of samples was done. This ratio was taken onto the linear calibration curves traced for each standard solution. Heavy metal analysis showed the arsenic level below the detection limit. The lead was found in the concentration of 0.15 µg/ml,whereas copper was found in the concentration of 0.17µg/ml, inAN, but both were of negligible quantity, so they could not be considered as a contaminant.

3.4. Pesticide content:

About 113 pesticides were tested including phorate, ediphenphos, myclobutanil, triazophos, tricyclazole, phosphamidon, butachlor, atrazine, Malathion, dimethoate, bifenazate, mandipropamide, azadiractin, spirodiclofen, malaoxon, etc. Myclobutanil was found in the concentration of 0.004µg/ml, for AN. All other pesticides were found to be absent in the sample.

4. Discussion:

Traditional herbal formulations play a significant role in effectively treating various diseases. The major challenge with herbal formulations is their complex chemical composition. Unlike synthetic drugs, the complexity of chemical constituents makes it difficult for developing suitable analytical methods for standardization. Markers-based standardization has been accepted as one of the useful techniques for the standardization of polyherbal formulation. AN is the herbal formulation consisting of herbal plant ingredients which have multiple proven functions such as memory enhancing, anti-inflammatory and antioxidant properties. Ayurveda, the prehistorically Indian medical science based on herbal remedies, is broadly admired for its global acceptance and uniqueness as it naturally treats diseases and promotes health management. The major challenge with standardization of herbal formulations is their complex chemical constituents hence there is always uncertainty about their safety and efficacy[21]. Unlike synthetic drugs, the complexity of chemical constituents makes standardization of Polyherbal formulations extremely difficult. Further, the lack of availability of chemical markers made this task much more difficult. As per the literature survey, a study reported mentioning the

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1511-1516 | ISSN:2251-6727

phytochemical and physiochemical parameters of *AN*. The physicochemical parameter revealed that the prepared in-house *AN* formulation complied with standards defined in protocols.

Physico-chemical parameters such as refractive index, viscosity, iodine value, saponification value, acid value, peroxide value, and free and total fatty acid contents were determined for *Acasia nilotica* using the standard protocols. The refractive index is the measure of the bending of a light ray when passing from one medium to another. It can also be defined as the ratio of the velocity of a light ray in a space to the velocity of light in a substance. The refractive index of *AN* formulation was determined as 1.31 at 40˚C. The acid value is used to determine the amount of free fatty acids in the fats. In this present study, AN in-house formulation showed a significantly lower acid value as compared to the reported *AN* formulation as per the *herbal* formulation[22].Thesaponification values are highly significant measures such that the saponification value is just right, if too high the soap might contain too much alkali even though there is sufficient soapiness that it would react with skin whilst a saponification value is too small, the fatty acid salts will not be sufficient enough to remove or saponify the fat or oil. The saponification value of in-house *AN* was found at optimal values of 157.57. The most important application of the iodine value is to determine the amount of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. The higher the iodine value, the more unsaturated fatty acid bonds are present in fat, here iodine value was obtained as 32.48 wt/ml. Peroxide is an indicator of the quantity of oxygen, as peroxide, in a particular substance. The value of the peroxide is an indication of the degree of oxidation present. The peroxide value for the *AN* formulation was reported as 5.42.

The congealing temperature is the point at which there exists a mixture of the liquid (fused) phase of a substance and a small but increasing proportion of the solid phase. It is distinct from the freezing point which is the temperature at which the liquid and solid phases of a substance are in equilibrium. In certain cases, this may happen over a range of temperatures. The congealing point of *AN* formulation lay between 20-17. Microbial content is measured for the presence of bacteria, fungi, and parasites. Even bacteria, fungi, and parasites have been found in herbal medicines, mainly because the raw material is exposed to microbial contaminants before harvesting and during handling and storage. Biological contamination can occur at any stage of drug production. Contaminants can be the result of the environment in which the medicinal plants are grown, the conditions under which they are dried and processed, the storage and transport conditions, or the manufacturing processes for ready-made medicinal products[21,]. In this present study, the prepared *AN* formulation did not show any microbial contamination. Mineral oil is the major source of our liquid fuels and petrochemicals[22]. It consists of long straight-chain alkanes and is contaminated by compounds containing sulfur, nitrogen, and metals. The mineral oil content was absent in the *AN* formulation.

Nowadays the analysis of polyphenols and also the flavonoids present in medicinal herbs has gained importance due to its correlation as anti-oxidants. So, because of this, it could be beneficial in therapies for neurodegenerative disease, cancer, and diabetes mellitus [23]. In the present study, the high TPC and TFC estimated in *AN* highlight its use in the management of oxidative stress. However, polyphenols present in the extract may bind with heavy metals present in *AN* because of their complex nature. These compounds after ingestion may get metabolized in the body leaving heavy metals inside the body or polyphenols may also act as a barrier for the transportation of heavy metals and may result in toxicity[24]. Hence in the present study, heavy metal analysis was carried out, and the results showed that arsenic and lead levels below the detection limit were determined using highly sensitive ICP-MS[25]. Copper was found in lesser concentration, but it was within the limits accepted, so it could not be considered a contaminant. Pesticides are used during the cultivation period of the plant. Some traces of pesticides are found after washing plants or plant parts, hence for the safety purposes pesticide the content was analyzed. The results of pesticide analysis revealed only traces of mycobutanil, but well within the accepted range. The absence of pesticides and heavy metals in the final AN formulation suggests that it is safe for internal use.

5. Conclusion:

Acacia is one of the popular formulations in which ghee is used as the main ingredient to extract lipid-soluble substances from the plants. Oil is mostly composed of fat and may increase the prevalence of coronary heart disease therefore needs monitoring before proving its efficacy. The present research work was carried out to establish an alternative method of extraction of *AN* and evaluate the quality control profile respective to the physicochemical properties, TPC, and TFC with the absence of pesticide and heavy metal residues, deeming it fit for internal consumption.

6. References:

1. G.E. Wickens. (1995). Role of *Acacia* species in the rural economy of dry Africa and the Near East.1st ed.; Food and Agriculture Organization of the United Nations: Rome, Italy, pp. 1-137.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1511-1516 | ISSN:2251-6727

2. A. Sala, M.D. Recio, R.M. Ginner, S. Manez, H.Tournier, G. Schinella and J.L. Rios. (2002). Antiinflammatory and antioxidant properties of*Helichrysum italicum*. Journal of Pharmacy andPharmacology. 54(3) 365-371
3. A. Sofowara. (1993). Medicinal plants andtraditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria: pp. 289.
4. J.B. Harborne. (1963). Plant polyphenols: X.Flavone and Aurone glycosides of *Antirrhinum*.Phytochemistry. 2: 327-334.
5. A. Banso. (2009). Phytochemical and antibacterialinvestigation of bark extracts of *Acacia nilotica*.Journal of Medicinal Plants Research. 3(2): 82-85
6. Adhikesavan, C., Rajagopalm, K. and SelwinRajaduari, J. (2015). Production and characterization of biodiesel from Acacia nilotica seeds. *International Journal of ChemTech Research*,**2**: 854- 859
7. Aldo, O., Temu,A. K., Ogwok, P. and Ntalikwa, J.W.(2012). Physico-chemical Properties of Biodiesel from Jatropha and Castor Oils. *International Journal of Renewable Energy Research*, **2 (1)**:379-384.
8. American Society for Testing and Materials (ASTM) (2000). Reapproved. Standard test methods for flash point by PenskyMartens closed up tester. D 93. ASTM
9. American Society for Testing and Materials (ASTM) (2000). Reapproved. Standard test method for cloud point of petroleum products. D 2500. ASTM.
10. American Society for Testing and Materials D 6751.Standad specification for biodiesel.
11. AOAC International (2000) Official Methods of Analysis of AOAC International 17th edn (Gaithersburg, MD: AOAC International)
12. Atabani, A., Silitonga, A.,Badruddin, I. A., Mahlia, T., Masjuki, H. and Mekhilef. S. (2012). A comprehensive review on biodiesel as an alternative energy resource and its characteristics.Renewable and Sustainable. Energy Review,**16:**2070–2093.
13. Atapour, M., Kariminia, H. R. and Moslehabadi, P. M. (2014). Optimization of biodiesel production by alkali-catalyzed transesterification of used frying oil. *Process Safety Environmental Protoco*l, **92**: 179–185.
14. Balakrishnan, K., Olutoye, M. A and Hameed, B. H. (2013). Synthesis of methyl esters from waste cooking oil using construction waste material as solid base catalyst. *Bioresources Technology*,**128:** 788–791.
15. Gaby, W. and Peter, S. L. (1997). Boiling point Properties and thermal Decomposition of Vegetable Oils Methyl esters with regard to their fuel stability. *Journal Agric Food Chemistry*. **45**: 4748- 4752.
16. Gastroprotective effect of *Acacia nilotica* young seedless pod extract: role of polyphenolic constituentsAsian Pac. J. Trop. Med.**(2012)**
17. D. Prakash, B.N. Singh and G. Upadhyay. (2007).Antioxidant and free radical scavenging activitiesof phenols from onion (*Alliums cepa*). Food Chemistry. 2: 1389-1393
18. R.S. Beniwal, O.P. Tokyo and P.K. Sharma.(2008). Effect of mycorrhizal fungi and phosphoruson growth and nodulation of *Acacia nilotica*. Journal of Agricultural and Food Chemistry. 5:172-176.
19. D. Fireston. (1997). Official methods andrecommended practices of the american oil chemists society (AOCS). 5th ed.; Champaign,AOCS Press: Boulder Urbana IL, United States ofAmerica, pp. 1-150.
20. J.B. Harborne. (1963). Plant polyphenols: X.Flavone and Aurone glycosides of *Antirrhinum*.Phytochemistry. 2: 327-334.
21. J.B. Harborne. (1963). Plant polyphenols: X.Flavone and Aurone glycosides of *Antirrhinum*.Phytochemistry. 2: 327-334.
22. R.S. Beniwal, O.P. Tokyo and P.K. Sharma.(2008). Effect of mycorrhizal fungi and phosphoruson growth and nodulation of *Acacia nilotica*.Journal of Agricultural and Food Chemistry. 5:172-176.
23. AbdErahman A, Abayomi OO, Ahmed AE, Nour AH, RbM Y, Ibrahim GM, Kabbashi NA (2018) Comparative analysis of polyphenolic and antioxidant constituents in dried seedlings and seedless *Acacia nilotica* fruits. J Anal Test 2:352–355
24. Abdoul GLB, Traore A, Ouedraogo M, Belemlilga M, Traore TK, Belemnaba L, Ouedraogo N, Lupu A, Ouedraogo S, Guissou IP (2019) Pharmacological study of trunk bark of *Acacia nilotica* var. adansonii (Guill et Perr). o Ktze (Mimosaceae): assays, antioxidant and antispasmodic activities. J Drug Deliv Ther 9:524–530

7. Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.