Comparison of *in-vitro* antimicrobial activities and selected properties of *Acacia nilotica* seed extract and its formulated oral capsules dosage form

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ABSTRACT

Background: The antimicrobial properties of *Acacia nilotica* seeds extract (ANSE) has been demonstrated in few studies. This property may be affected when the extract is formulated into modern dosage form which involves a number of formulation processes.

Objectives: This study was carried out to evaluate and compare the *in-vitro* antimicrobial activities of ANSE and its formulated capsules against some commonly implicated organisms in the gastrointestinal tract.

Methods: Seeds of *Acacia nilotica* obtained from Ilorin were prepared and extracted with absolute methanol. An oral capsule formulation was designed for the extract. *in-vitro* antimicrobial activities of the extracts and formulated capsules were carried out on four test organisms including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans.* This was done using the pour plate method. Physical properties and the flow properties of both the crude extract and formulated capsules were also determined and compared using official standards.

Results: There was no marked difference between the colour, taste and odour of ANSE and formulated granules for oral capsules. There was a decrease in moisture content and pH of ANSE after formulation into granules. The flow properties of the crude extract revealed a poor flow which improved after extract was formulated into granules. The methanol extract exhibited antimicrobial activities against test organisms pre- and post-granulation/ encapsulation and activity was not significantly affected by the encapsulation processes ($p>0.05$).

Conclusion: Granulation of ANSE before encapsulation improved its flow properties. The formulation of ANSE into oral capsule dosage form did not significantly affect its antimicrobial activities.

Keywords: *Acacia nilotica* seed extract, Oral capsules, antimicrobial activities, physical properties, flow properties.

Comparaison des activités antimicrobiennes *in vitro* et de certaines propriétés de l'extrait de graines *d'Acacia nilotica* et de sa formulation en capsules orales

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RÉSUMÉ

Contexte: Les propriétés antimicrobiennes de l'extrait de graines *d'Acacia nilotica* (ANSE) ont été démontrées dans quelques études. Cette propriété peut être affectée lorsque l'extrait est formulé sous une forme de dosage moderne, ce qui implique un certain nombre de processus de formulation.

Objectif: Cette étude vise à évaluer et comparer les activités antimicrobiennes *in vitro* de l'ANSE et de ses capsules formulées contre certains organismes couramment impliqués dans l'appareil digestif.

Méthode: Des graines *d'Acacia nilotica* obtenues dans la ville d'Ilorin ont été préparées et extraites avec du méthanol absolu. Une formulation orale en capsule orale a été conçue pour l'extrait. Les activités antimicrobiennes in vitro des extraits et des capsules formulées ont été menées sur quatre organismes testés, à savoir *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa et Candida albicans*. Cette étude a été réalisée à l'aide de la méthode de la plaque de coulée. Les propriétés physiques et les propriétés d'écoulement de l'extrait brut et des capsules formulées ont également été déterminées et comparées en utilisant les normes officielles.

Résultats: Il n'y avait pas de différence marquée entre la couleur, le goût et l'odeur de l'ANSE et des granulés formulés pour les capsules orales. La teneur en humidité et le pH de l'ANSE ont diminué après la formulation en granulés. Les propriétés d'écoulement de l'extrait brut ont révélé un faible écoulement qui s'est amélioré après la formulation de l'extrait en granulés. L'extrait méthanolique a présenté des activités antimicrobiennes contre les organismes testés avant et après la granulation/encapsulation et l'activité n'a pas été affectée de manière significative par les processus d'encapsulation ($p>0,05$).

Conclusion: La granulation de l'ANSE avant l'encapsulation a amélioré ses propriétés d'écoulement. La formulation de l'ANSE sous forme de capsule orale n'a pas affecté de manière significative ses activités antimicrobiennes.

Mots-clés: Extrait de graines *d'Acacia nilotica,* capsules orales, activités antimicrobiennes, propriétés physiques, propriétés d'écoulement.

INTRODUCTION

There have been records of advances made in modern/ synthetic medicines but there are still many ailments or infection for which suitable drugs are yet to be found. This has brought an urgent need to develop safer drugs (both for man and his environment) for the treatment of various ailments including microbial infections of various causes among others. Through recent research on herbal plants or medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine. Consequently, plants can be described as a major source of medicines, not only as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population. Modern medicines and herbal medicines are complementarily being used in areas for health care programmes in several developing countries such as countries in Africa, Asia and some parts of Europe. Due to different outcomes on herbal plants, plant products are used all over the world with the belief that many herbal medicines are known to be free from health and environmental effects. The fear of the masses in the utility of synthetic drug or modern drugs is always accompanied by their single or multiple adverse or health effects.[1]

Many countries in the world depends on herbal medicine for primary health care because of acceptability, better compatibility and adaptability with the human body and poises lesser side effects. Herbal medicines proved to be the major remedy in the traditional system of medicine. They have been used extensively in medical practices since ancient times. This prompted the development of the practices of medicinal plants. The reasons are because of their biomedical benefits as well as their place in cultural beliefs in many parts of the world in the development of potent therapeutic agents.[1]

Acacia is a genus belonging to the *Fabaceae* family; *Fabales* order; *Rosidae* subclass; *Magnoliopsida* class, *Magnoliophyta* division; *Spermatophyta* Super division; *Tracheobionta* subkingdom and Plantae Kingdom[2]. Research works have been carried out on the various parts of the Acacia nilotica including its seeds for various purposes and uses. This study however, focuses on its antimicrobial properties. Different studies have been carried out to determine its antimicrobial properties using a wide number of organisms including *Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Escherichia coli,* to mention a few and all the experiments proved that different parts of the plants including the seeds have significant antimicrobial properties against all the organisms studied.[3-10] Acacia nilotica has been widely studied, especially for its antimicrobial properties but with limited modern dosage formulations. Two studies have attempted to formulate the seed extract of this plant into cream and microcapsules.[2,10] This necessitates the need to study further other common modern solid and liquid dosage forms.

Despite the recent advancement in herbal medicine, one of the most difficult issues to contend with in translating traditional herbal practices into conventional orthodox medicine. One of these problems is the lack of information on the social, biochemical and economic benefits that could be derived from the industrial utilization of medicinal plants. In addition, there are few incentives for the standardization of products and little information on the market potential and trading possibilities of these medicinal plants. These result in underutilization or less exploitation of the real potential of these plants.[1] Synergizing herbal medicines with modern dosage formulation through research works is therefore of utmost importance to create more acceptability among people who believes in modern medication, assist in controlling the dosage and usage as well as enhance the various benefits embedded with herbal products. This study thus, aims at formulating Acacia nilotica seed extract (ANSE) into oral capsule dosage form and to compare the antimicrobial activities and some physicochemical properties of the crude seed extract with the formulated capsules.

METHODS

Collection of Materials

The dried seeds of Acacia nilotica were obtained from Ilorin, identified, authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, assigned voucher number ULLH/001/1174/2022 and kept in the herbarium.

The dried seeds were removed from the pods, pulverized and sieved using the 600 µm mesh size to obtain uniform particle size. The sieved material was packaged in an airtight container and stored in a desiccator for further use.

Extraction of Dried Seed Extracts

Ten grams (10 g) of the dried pulverized and sieved Acacia nilotica seeds were macerated and thoroughly shaken thrice daily in 100 ml of absolute methanol (BDH Analar, England) for 72 hours. The resulting mixture was filtered

using filter paper through a Vacuum pump (VE 160N, Value, India). The supernatant thereafter obtained was concentrated over a water bath (SM-8A, Surgifield Medical, England) at 40°C. The weight of the resulting dried product was determined on an analytical balance (Pioneer, Ohaus, Switzerland) and thereafter packaged in an airtight container and kept in a desiccator.

Formulation of ANSE granules and oral capsules

The wet granulation method was used for granules formulation. The dosage of the extract was calculated by multiplying the pre-determined MIC of the extract (20 mg/ml) and average gastric volume at rest of 35 ml as obtained from the previous study.[12] This gave 700 mg of extract per single dose. This study was thereafter designed to contain 500 mg of granules (containing 350 mg of the extract) per capsule to allow the usage of two capsules for a single dose.

A batch size of 150 capsules was adopted for this study and the procedures used for production are as follows:

maize starch (15.75 g) and the same quantity of Acacia nilotica seed extracts were weighed and mixed in a mortar and pestle and triturated for 15 minutes until a uniform mix was obtained. The remaining quantity of the plant extract (36.75 g) was added to the mixture and trituration was continued for another 15 minutes until an even mix was obtained.

Maize starch (0.525 g) was weighed into a 100 ml beaker while the paste was made by adding 10 ml of deionized water and mixed using a stirrer. Afterwards, about 100 ml of deionized water was boiled on an electric heater. Fifty (50) ml of the boiled water was accurately measured and added gradually into the mixture obtained while mixing until a viscous mixture was obtained.

The resulting two mixtures (Acacia nilotica mixture and paste mixture) were mixed and blended for 15 minutes. The resulting damp mixture was passed through a mesh size 8 using a granulating machine (MM3HP, Mec-Well, India) and dried in a tray dryer (TD43, Mec-Well, India) for 20 minutes at 70^{o}C.

The dried powder was thereafter passed through mesh size 4 using a granulating machine (MM3HP, Mec-Well, India) to obtain an even particle size. Talc and Magnesium stearate (0.75 g each) were added to the granulated powder and further mixed for 10 minutes. The hard gelatin capsules (size 0) were then filled with 500mg of the granules using an Encapsulating machine (CTF-300, Captech Systems, India). The product was stored in an airtight container away from light and moisture for further use and analysis.

Control capsules were produced. The same composition used for the ANSE capsules was also adopted. The difference however, was that ANSE was removed and substituted with same quantity of lactose for the control capsules.

Physical properties of ANSE and its formulated granules for oral capsules

Physical properties determined included appearance, taste, odour and colour using the respective sensory organs.

The pH of a solution of 1 mg/ml of the extract and the formulated granules were prepared in Phosphate buffer (pH 7.0) and determined using a pH meter (3510, Jenway, UK) by following the Standard Operating Procedures and manufacturer's instructions for the Instrument. The pH value displayed on the instrument was thereafter recorded.

The moisture content of 10 g each of the ANSE and formulated granules (were done using the moisture analyzer (MB45, Ohaus, Switzerland) by following the Standard Operating Procedures and manufacturer's instructions of the instrument. The moisture content value displayed on the instrument was thereafter recorded.

Flow Properties of ANSE and granules for oral capsules

Bulk Density, Tapped densities, Hausner's ratio and Carr's Index

***Acacia nilotica* seed methanol extract:** An amount (10 g) of was weighed and poured through a funnel into a 100-tarred measuring cylinder. The cylinder was then lightly tapped twice to collect all the granules sticking on the wall of the cylinder. The initial volume, V0 was recorded. The cylinder was tapped from a height of 2.5 cm, 50 times on a wooden bench top to attain a constant volume reading from the cylinder, Vf

The initial density was calculated as the initial bulk density, D0:

D0 = mass/ V0

The final density was also calculated as the final bulk

density or tapped density, Df:

Df = mass/ Vf

The ratio Df/ D0 was calculated as Hausner's ratio.

Carr's Index also known as percentage compressibility was calculated as Df - D0/Df x 100.

The same procedure was repeated for formulated granules for oral capsules.[5,6]

Angle of Repose

This was determined by using the fixed funnel, fixed height method. A 50 g quantity of the extract was allowed to flow freely from the funnel at a distance of 3 cm from the tip of the funnel to the horizontal surface to form a cone. The base of the cone was marked and the pile of powdered extract was also poured off. The average of the two diameters was determined. The angle of repose (Ø) was then calculated from the height of the cone (h) and the calculated average diameter (d) of the marked base of the cone from the relation:

Ø = tan-1 (2h/d)

A similar procedure was repeated for the formulated granules for oral capsule.[13,14]

Screening for antimicrobial activity of ANSE and the formulated oral capsules

The ANSE capsules were screened for antimicrobial activity using the Agar cup plate method.

Twenty ml (20 ml) of molten nutrient agar (Sabourand Dextrose Agar for Candida albicans) was poured into each Petri dish and was allowed to set. The surface of the agar was then flooded in with standardized cultures of the test organisms (Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25913), Pseudomonas aeruginosa (ATCC 27853), and Candida albicans (Clinical isolate) previously diluted to give approximately 106cfu/ml. The excess culture on the surface of the agar was then drained off.

Various concentrations of ANSE were prepared with Dimethyl sulphoxide (DSMO) used as the suspending medium. Four holes (cups) were made on each petri dish with the aid of a sterile cork-borer (size 4) such that the following concentration was introduced into each cup in every Petri dish: the first cup contained 0.1 ml equivalent of 20 mg/ml of ANSE in capsule solution; second cup contained 0.1 ml equivalent of 40 mg/ml of ANSE in capsule solution, third cup contained 0.1 ml equivalent of 40mg/ml of lactose in control capsule solution and fourth cup contained 0.1 ml of DMSO. The above procedures were done in duplicate for each organism used.

The solutions were then allowed to diffuse for one hour and then incubated at 37^{o}C for 24 hours for the bacterial organisms and 25^{o}C for 48 hours for Candida albicans. The zone of inhibition of the various solutions for each Petri dish was then measured with a vernier calliper. Each zone of inhibition was obtained by measuring the largest and smallest diameter of each zone of inhibition and recorded.

The same procedures were followed using ANSE.

Statistical Analysis

Data obtained were analyzed using SPSS version 20. Results were presented in the form of tables, percentages, mean ± standard deviation and graphs. Inferential statistics done was a t- test analysis with a p-value < 0.05 considered statistically significant.

RESULTS

Physical properties of ANSE and its formulated granules

The seed extract showed a shiny dark brown powder with a bitter taste and a characteristic odour. Moisture content was 6.19 % and pH was 7.0.

The formulated granules were coarse dark brown with bitter taste and a characteristic odour. The moisture content was 2.64 % and the granules had a pH of 6.8.

Table 1: Flow properties of ANSE and its formulated granules

Properties	ANSE	Formulated granules of ANSE
Bulk density (g/ml)	0.595	0.625
Tapped density (g/ml)	0.813	0.714
Hausner ratio	1.366	1.142
Carr's index	26.80	12.46
Angle of repose	40.60	33.69

Table 1 shows the various flow properties of the methanol extract and formulated granules of Acacia nilotica seeds. The Hausner ratio and Carr's index for ANSE were generally higher than that of the formulated granules while the formulated granules had a higher angle of repose compared with that of ANSE.

Table 2: Antimicrobial properties of ANSE and formulated capsules

Organisms	**Mean zone of inhibition ± SD (mm)**					
	Extract (20mg/ml)	**Extract (40mg/ml)**	**Capsule (20mg/ml)**	**Capsule (40mg/ml)**	**Control 1 (Lactose cap)**	**Control 2 (DMSO)**
E. coli	17.50 ± 0.58	18.25 ± 0.50	18.50 ± 0.58	19.00 ± 0.00	0	0
S. aureus	19.75 ± 0.50	19.50 ± 0.58	19.00 ± 0.00	19.25 ± 0.50	0	0
Ps. aeruginosa	18.75 ± 0.50	19.00 ± 0.82	17.50 ± 0.58	18.50 ± 0.58	0	0
C. albicans	18.00 ± 0.00	18.75 ± 0.50	18.00 ± 0.82	18.75 ± 0.96	0	0

The Antimicrobial properties of the formulated ANSE capsules are stated in Table 2. At 20mg/ml, both the extract and the capsule exhibited the highest zone of inhibition against S. aureus when compared to other organisms used. Similar results were obtained for the 40mg/ml extract and capsules. The 20mg/ml extract had the lowest zone of inhibition against E. coli while the 20mg/ml capsule had the lowest inhibition against Ps. aeruginosa.

Table 3: Comparison of zones of inhibition of ANSE and its formulated capsules using t-test analysis

Test Organisms	**Extract vs. Capsules (20mg/ml)**		**Extract vs. Capsules (40mg/ml)**	
	t-value	**p-value**	**t-value**	**p-value**
S. aureus	3.0000	0.0577	0.5222	0.6376
E. coli	2.4495	0.0917	3.0000	0.0577
Ps. aeruginosa	2.6112	0.0796	1.7321	0.1817
Candida albicans	0.0000	1.0000	0.0000	1.0000

Table 3 compares the zones of inhibition of the extract and the formulated capsules at two different concentrations (20mg/ml and 40mg/ml) using the t- test analysis. The Table shows that all the p values obtained for the various organisms at the two concentrations were above 0.05 showing that it is not statistically significant.

DISCUSSION

The tests conducted to determine the physical properties of the methanol extract and ANSE granules show that the granules formation process did not affect the extract significantly. The colour, taste and odour were similar pre and post-granulation. However, the moisture content of the formulated granules (2.64 %) was lower than the raw extract (6.19 %). This is a result of the processes involved in granules formulation especially the drying process of the granules tends to reduce or control the moisture content of the granules. The water composition of plant material or extract has been found to affect its physicochemical properties.[15] High moisture content promotes degradation and spoilage. It is therefore desirable to reduce the moisture content of a formulation to reduce or prevent easy deterioration of the drug. The moisture content obtained in this study for both extract and granules falls within the acceptable limit between 8 and 10% for vegetable drugs as stated in the African Pharmacopoeia.

Similarly, the pH of the formulated granules (6.8) was lower than the raw extract (7.0). The addition of different excipients during the formulation of the granules can alter the pH of the original extract since these excipients have their pH that can impact the final pH of the formulated granules. The pH has been found to influence the nature and type of formulation among other factors.[16] Similar observations regarding colour, smell, taste, moisture content and pH were made in a similar study where encapsulation of plant extract was studied.[17] It should be noted that the dissolution medium did not impact the pH of the substance under study since the buffer solution used was corrected to a neutral pH of 7.0.

The flow properties of the methanol extracts as well as the formulated granules are presented in Table 1. The various values obtained for the methanol extract of ANSE revealed that the extract pre-granulation had poor and cohesive flow when calculated values are compared with the official standard values. The Hausner ratio of 1.366 falls within the official range of 1.35 to 1.45 which is interpreted as poor flow. The calculated Carr's index of 26.8 also falls into the official poor flow range of between 26 and 3118. Similarly, the interpretation of the value for the angle of repose obtained (40.6) shows that the powder is poor and must agitate or vibrate (Official range of 41 to 45) (Aulton and Taylor, 2017). In general, it can be said that the flow property of the raw extract needed to be improved before encapsulation to promote good flow.

The flow property of the extract post-granulation however, showed significant improvement over the result obtained pre-granulation. The Hausner ratio reduced to 1.142 which falls within the official range of 1.12 to 1.18 indicating a good and free flow of powder; the Carr's index also reduced from 26.8 to 12.46 agreeing with the official range for good and free-flowing powder (official range of 11 to 15) and the angle of repose gave a value of 33.69 which similarly falls in the official range of 31 to 35 interpreted as good flowing powder[14]. These results show that the granulation process of the extract helped to improve the flow of the powdered extract to enhance easy encapsulation. Components such as binders, lubricants and glidants incorporated during the encapsulation process aided in improving the flow of the formulated granules. Good flow properties for granules improve uniformity in filling and ease of encapsulation.[13,19] A poorly flowing powder may become sticky which leads to uneven powder flow through the hopper, this may in turn affect factors like weight uniformity of capsules and caking tendencies on storage. Similar studies on the encapsulation of plant extracts made similar observations as obtained from this study regarding the flow properties of extract pre- and post-granulation.[11,20]

The general objective of this study focused on the antimicrobial properties of the extract of A. nilotica. This was carried out pre-granulation/ encapsulation and post-granulation/ encapsulation as presented in Table 2. The result shows that as low as the concentration of 20mg/ml of the extract and the formulated capsules, activities were recorded against all the various organisms used for this study. The zone of inhibition was highest against *S. aureus* when tests were done on extract and formulated capsules. A significant zone of inhibition was also observed against other organisms. The result also obtained from Control 1 and Control 2 clearly shows that excipients used in the production of the formulated capsules had no influence on the various zones of inhibitions obtained and similarly DSMO which was used as the dissolving medium for the extract and granules also had no antimicrobial property. The result obtained in this study is similar to the one reported in a similar study where it was discovered that microcapsules of ANSE exhibited significant zones of inhibition against various test organisms.[11]

Inferential statistics using the t-test analysis was used to compare the zones of inhibition obtained from the raw

extract as well as the formulated capsules (Table 3). The result clearly shows that all the p-values calculated are above 0.05 (with significance set at 95%) which translates to the fact that there is no statistical significance between the zones of inhibition obtained against the various organisms when the raw extract was used compared to when the extract was formulated into ANSE capsules. Invariably, the antimicrobial actions of ANSE are not affected by excipients and processes involved in formulating it into granules and capsules. Therefore, the capsule dosage form of ANSE may serve as a potential antimicrobial agent.

CONCLUSION

The ANSE capsules were successfully formulated, and they demonstrated promising antimicrobial activities and therefore, could be explored as antibiotic, especially in gastroenteritis where one or more of the interrogated organisms could be implicated.

The authors report that there are no competing interests to declare.

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