

Original Research Article**IN VITRO ANTIMICROBIAL EFFECT OF CRUDE TANNINS ISOLATED FROM****THE LEAF OF ANNONA SENEGALENSIS****ABSTRACT**

Antimicrobial effect of tannin isolated from the leaf of *Annona senegalensis* on *Shigella dysenteriae*, *Escherichia coli* and *Salmonella typhi* was investigated. The phytochemical screening of the leaf of the plant revealed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides and anthraquinones while phenols and steroids were absent. Quantitatively, tannins were found to be 24% in the leaf of the plant. Isolated tannin exhibited antibacterial activities against all the tested bacteria; *Shigella dysenteriae* gave the zone of inhibition (18.6mm), *Escherichia coli* (13.9mm) and *Salmonella typhi*(10.6mm). Minimum Inhibitory concentration of the crude tannins on the test organisms ranged from 6.25mg/ml to 12.5mg/ml. These findings confirmed the basis of traditional use of the leaf of *Annona senegalensis* in the treatment of diseases such as dysentery and diarrhea.

KEY WORDS: Antimicrobial, Phytochemicals, Zone of inhibition, and Crude tannins.

INTRODUCTION

Medicinal plants are cheap starting materials for the synthesis of new drugs and the chances of the body accepting preparations from plants are more than that prepared from laboratory (Sofowora, 1988). Also, Fansoworth (2001) has reported that aqueous decoction of drug has a greater bioavailability to the body than synthetic formulation today in the market. This is due to the fact that presentation of the active constituent of the plant material in the solubilized form brings about increase absorption of the extract by the body.

In recent years, the interest to evaluate plants possessing antibacterial activity for various diseases is still growing (Clark and Hufford, 1993). Herbal Medicine is still the main stream of about 75 to 80% of the whole population and the major part of traditional therapy involves the use of plant extract and there active constituents (Acharya *et al.*, 2008). Some plant has also been reported to exhibit antimicrobial activities against various pathogen (Oladunmoye, 2006).

Annona senegalensis commonly known as African custard apple is an important plant that is used in traditional medicine to cure some diseases like toothache and diarrhea. The leaf of the plant is used for treatment of cancerous tumor, intestinal trouble and venereal disease. It is used for astringent and for treating dysentery and malaria (Diallo and Paulsen, 2000). Locally, the plant is known as “Gwandar daaji” in Hausa, “UburuOcha” in Igbo and Abo in Yoruba (Alqasim, 2013). The part of the plant that is used for therapeutic purposes include root, bark, leaf, flower and seed.

Tannins are polyphenols that are obtained from various parts of different plants (Gajendiran and Mahadevaan, 1990). In addition to use in leather processing industries, tannin have shown potential antiviral (Lin *et al.*, 2004), anti bacterial (Akiyama *et al.*, 2001; Funatogowo *et al.*, 2004), and anti parasitic effects (Bhagavalli *et al.*, 1999). In the past few years tannins have been studied for this effect against cancer through different mechanisms (Sowemimo *et al.*, 2007).

This work is designed to extract crude tannin from the leaf of *Annona senegalensis* and to evaluate its efficacy ~~or otherwise~~ on causative organisms of the dysentery.

MATERIALS AND METHODS

Collection of Plant Material

Formatted: French (France)

Formatted: French (France)

Fresh leaves of *Annona senegalensis* was collected in Sangere under Girei Local government Area of Adamawa state. It was identified and authenticated in the department of forestry, school of Agriculture ~~And~~ Agricultural Technology, Modibbo Adama University of Technology, Yola.

Sources of Test Organisms

The microorganisms *Shigella dysenteriae*, *Salmonella typhi* and *Escherichia coli* were obtained from the Department of Microbiology, Modibbo Adama University of Technology, Yola.

Preparation of the Extracts

Exactly 50g of the powdered sample was weighed and transferred into 500ml of distilled water in a conical flask. This was allowed to stand for 24 hours at room temperature. The suspension was filtered using sterile what man No. 1 filter paper to obtain crude extract. A rotary evaporator was used to concentrate the extract. The concentrated extract obtained was stored in a refrigerator in a screw-cap bottle until when it was required.

Phytochemical Analysis

Chemical tests was carry out on the aqueous extracts to identify the constituents as described by Sofowora (1981), Trease and Evans (1989).

Extraction and Quantitative Determination of Tannins

The dry leaf was ground and 100mg of the fine powdered portion was dissolved with 1ml of concentrated HCl in methanol (1:100, v/v) in a water bath at 30°C for 30mins and was shook for every 5 minutes. The sample was centrifuged at 1200g and the supernatant was collected. Extraction was repeated twice; the combined supernatant was then filtered, evaporated to dryness and the weight was taken. The extract was kept for the antimicrobial test (Morrison *et al.*, 1999).

Preparation of Culture Medium

Comment [SM1]: No need for this section.

Nutrient agar was used as medium; 14g of nutrient agar was weighed and transferred in to a flask. In the flask, 500ml of sterile distilled water was added to the nutrient agar and gradually stirred. The mouth of the flask was covered with cotton aid wrapped with aluminum foil. This was sterilized in an autoclave at 121⁰C for 15 minutes. After sterilization, the nutrient agar was stored in a refrigerator for future use.

Antimicrobial Activity Testing

Wells were made on the surface of 20ml nutrient agar plates previously streaked with test organisms using ~~1-00.5~~ McFarland standard (3×10^8 organisms). Extract of 0.2ml was aseptically introduced into the wells made; 100mg of ciprofloxacin was used as positive control which was added into a separate well. The plates were allowed to stand on the working bench for 30minutes and were incubated for 24hours at 37⁰C in an incubator. The presence of zone of inhibition was regarded as the presence of antimicrobial activity and this was expressed as the length of the diameter of the zones of inhibition (Emeruwa, 1982).

Comment [SM2]: 100mg is too much, ciprofloxacin is used in 1-5 microgram disks !!!!

Comment [SM3]: Add the diameter of the well, please.

Comment [SM4]: You should use recent reference.

Determination of Minimum Inhibitory Concentration (MIC)

Different concentrations of tannins from the leaf of *Annona senegalensis* ranging between 12.5 and 6.25mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of *Salmonella typhi*, *Shigella dysenteriae* and *Escherichia coli* diluted to give a final concentration of cells per ml. The tubes were incubated at 37⁰C for 24hours. The least concentration of tannin that did not permit any visible growth of the incubated test organism in broth culture was regarded as the Minimum Inhibitory Concentration (MIC) in each case (Collins *et al.*, 1995).

110

111 RESULTS

112 The leaf of *Annona senegalensis* was found to contain tannins, alkaloids,
113 saponins, flavonoids, terpenoids, anthraquinone and cardiac glycosides. Phenols
114 and steroids were absent (Table 1).Quantitative determination of tannins yielded
115 24% in the leaf of the plant.

116 The crude tannin exhibited antibacterial activities against all the tested
117 bacteria; *Shigella dysenteriae* gave the zone of inhibition (18.6mm),
118 *Escherichia coli* (13.9mm) and *Salmonella typhi*(10.6mm) as shown in table 2.

119 Determination Minimum Inhibitory concentration (MIC)of the crude
120 tannins gave 6.25mg/ml on *Shigella dysenteriae* and 12.5mg/ml on both
121 *Escherichia coli* and *Salmonella typhi*. This is presented in table 3.

122

123

124

125

126

127

128

129 **Table 1: Phytochemical Screening**

Phytochemicals	Results
Tannins	++
Alkaloids	+
Saponins	+
Terpenoids	+
Phenols	-
Steroids	-
Anthraquinones	+

Flavonoids	++
Cardiac glycosides	++

Key: + = present, - = absent and ++ = highly present

Table 2: Antimicrobial activity of tannin isolated from the leaf of *Annona senegalensis*

Test Organisms	Zones of inhibition (mm)	
	Crude Tanins	Ciprofloxacin
<i>E.coli</i>	13.90	34.00
<i>S. typhi</i>	10.60	21.00
<i>S.dysenteriae</i>	18.60	26.00

Formatted: French (France)

Formatted: French (France)

Formatted: French (France)

Table 3: Minimum Inhibitory Concentration of Tannins Isolated from the Leaf of *Annona senegalensis*

Organisms	Concentrations (mg/ml)					
	50	25	12.5	6.25	3.0	1.5
<i>E. coli</i>	-	-	-	+	+	+
<i>S.typhi</i>	-	-	-	+	+	+

S.dysentriae - - - - + +

Keys: + = indicates growth and - = indicates no growth

DISCUSSION

Phytochemical analysis of the extract of leaf of *Annona senegalensis* revealed the presence of chemical constituents namely tannins, alkaloid, saponin, flavonoid, terpenoid, cardiac glycoside and anthraquinone. However, the presence of alkaloid and anthraquinone was in contrast with the work of Tijjani *et al* (2013), who reported that alkaloid and anthraquinone were absent in the leaf of the plant. The observed differences may be due to the different solvent used or where the plant is harvested or could be due to storage.

The various phytochemical compound detected are known to have beneficial medicinal value. There are established records that showed the benefits of these compounds detected in *Annona senegalensis*, for instance, tannins are reported to exhibit antiviral, antibacterial, antitumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic (Nonaka *et al.*, 1990).

Saponin in medicine, it is used in hypercholesterolaemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc. it is also known to have anti-fungi properties (Denwik, 2002).

Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergic and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Pengsuperpet *al.*, 1995).

Anthraquinones are generally used as dyes and are also known as antibacterial agent (Hemen and Lalita, 2012).

Comment [SM5]: Very extensive discussion. You need to concentrate on tannins.

166 Cardiac glycosides are used in the treatment of congestive heart failure.
167 They are also used to strengthen a weakened heart and allow it to function more
168 efficiently though the dosage must be controlled carefully, since the therapeutic
169 is close to toxic dose (Denwik, 2002).

170 Terpenoid are active against bacteria, fungi and viruses (Fujjoka and
171 Kashwada, 1996) and protozoa.

172 Moreover, these chemical constituent were reported to exhibit prominent
173 effect on human and other animal system through different mechanisms of
174 action which make them useful for medicinal purposes. For instance
175 anthraquinone are known to complex irreversibly with nucleophilic amino acid
176 in protein (Steinberg *et al.*, 1996). Often leading to inactivation of the protein
177 and loss of function. For that reason, the potential range of quinone
178 antimicrobial effect is great. Probable targets in the microbial cell are surface –
179 exposed adhesions, cell wall polypeptides and membrane bound enzymes.
180 Quinone may also render substrate unavailable to the microorganism.(Kazmi *et al.*, 2006).

182 The mode of antimicrobial action of tannins as described in
183 anthraquinone may be related to their ability to inactivate microbial adhesions,
184 enzymes, cell envelopes transport proteins. They also complex with
185 polysaccharide (Yao *et al.*, 1999). Tannins in plants inhibit insect growth
186 (Schltz, 1999) and disrupt digestion events in ruminant animal (Butler, 1999).

187 One of the molecular actions of tannins is to complex with protein
188 through so called non-specific forces such as hydrogen bonding and
189 hydrophobic effects, as well as by covalent bond formation (Scalbert, 2004;
190 Jones *et al.*, 2006).

191 Mechanism of action of alkaloids such as berberine and harmane is
192 attributed to their ability to intercalate with DNA / cell wall of an organism
193 thereby disrupting the activity of the microorganism(Hoppet *et al.*, 2003).

Formatted: German (Germany)

Formatted: German (Germany)

Formatted: French (France)

Formatted: French (France)

194 However, the mechanism of action of terpenoids is not fully understood
195 but is speculated to involve in membrane disruption by the lipophilic
196 compound. According to Mendez *et al*, (2000) found that increasing the
197 hydrophilicity of kaurenedi terpenoids by addition of a methyl group drastically
198 reduced their antimicrobial activity (Denwik, 2002).

199 Cardiac glycosides are known to work by inhibiting the Na^+/K^+ pump.
200 This causes an increase in the level of sodium ion in the myocyte which then
201 lead to a rise in the level of Ca^{+} . This inhibition increases the amount of Ca^{+} ion
202 available for contraction of the heart, muscles which improve cardiac output and
203 reduce distension of the heart (Denwik, 2002).

204 Crude tannins from the leaf of the plant were found to be 24% and this is
205 in agreement with the earlier study carried out by Simon (1993). However,
206 Bakar (2007) reported that tannin is very rich in fruit of *Annona senegalensis* than
207 in the leaf even though he has not mentioned the values.

208 The antimicrobial activity of tannins isolated from the leaf showed
209 highest activity against *Shigella dysenteriae* and moderate in *Escherichia coli*
210 and *Salmonella typhi* and ciprofloxacin was used as standard antibiotic as shown
211 in table 2 .

212 The sensitivity observed in this study is in agreement with earlier
213 investigation by ~~(Doss *et al.*, (2009) and (A. Kiyama *et al.*, (2001)~~. It was
reported by 214 Jone *et al.* (2006) that tannins has the ability to interfere with the
bacterial cell 215 wall synthesis where they formed complex with
polysaccharide through so 216 called non-specific forces such as hydrogen
bonding and hydrogen effects as 217 well as by covalent bond formation.
Thus, tannin irreversibly inhibits the 218 enzyme trans peptidase by reacting
with serine residue in the trans peptidase. 219 This reaction is irreversible and so
the growth of the bacteria cell is inhibited. 220 Also reported by (Viljoen *et al.*,
2003) the ability for the tannin compounds to 221 cause the bacterial colonies
to disintegrate probably results from their 222 interference with the bacterial
cell wall, thereby inhibiting the microbial growth.

Comment [SM6]: and

The minimum inhibitory concentration (MIC) of tannin isolated in this study against the test organism ranged between 6.25 and 12.5mg/ml as shown in table 3. Antimicrobial agent with low activity against an organism had a high MIC while a highly active antimicrobial agent gave low MIC. For instance *Shigella dysenteriae* has the highest zone of inhibition at the same concentration with other organism, but gave the low MIC. The antibacterial effectiveness with the increasing concentration of tannins observed in the present study is in agreement with the earlier investigation as reported by Kurosaki and Nishi (1983) and Banso and Adeyemo (2007). However, a minimum inhibitory concentration (MIC) is generally regarded as the most basic laboratory measurement of activity of an antimicrobial agent against an organism.

The clinical significance of Minimum Inhibitory Concentration (MIC) are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial agents. Applying MIC testing to number of bacterial strains in the same species provides an estimate of the concentration that inhibits 50% (MIC₅₀) and 90% (MIC₉₀) of bacterial isolates and can indicate shifts in the susceptibility of bacterial population to antibiotics (Davison, 2000).

In conclusion, the result of this study supports the traditional use of the *Annona senegalensis* for the treatment of diseases such as dysentery, diarrhea and typhoid. It also suggests that the tannin isolated from the test plant could serve as a potential source of antibiotics.

REFERENCES

Acharya, C.A. Deepak, K. and Shrivastava, A. (2008). Indigenous Herbal Medicine: Tribal Formulations and Traditional. Herbal Practices, Aavishkar Publisher Distributor, Jaipur – India. pp. 440.

- 250 Akiyama, H., Kazuyasu, F., Yamasaki, O., Oono, T. and Iwatsuki, K.
 251 (2001).Antibacterial action of several tannins against *Staphylococcus aureus*.*J.*
 252 *Antimicrobial Chemotherapy*. 48(48):487 – 491.
- 253 Alqasim, A.M. (2013). *Annonasenegalensis* person: A Multiposes Shrub, its
 254 *science and technology*. Volume 2, No.ISSN 2049- 7318.
- 255 Bakar, K. (2007). *In vitro* antioxidant studies in leaves of *Anonnaspecies*.
 256 *Indian Journal of Experiment Biology*. Vol. 45, pp. 480 – 485.
- 257 Banso, A. and Adeyemo, S.O. (2007). Evaluation of antibacterial properties of
 258 tannins isolated from *Dichrostachys cinerea*. *Afr. J. Biotechnol.* 6(15):1785–
 259 1787.
- 260 Bhagavalli, A., Narayanana, Otto Geoffroya, Mark, C., Willinghama, Gian, G.,
 261 Reb and Daniel, W. Nixona (1999). Expression and its possible role in G1 arrest
 262 and apoptosis in ellagic acid treated cancer cells. *Cancer Letters*. 136, 215 –
 263 221.
- 264 Butler, L.G. (1999). Effect of condensed tannin on animal nutrition in
 265 Hemingway R.W., Karchesy, J.J. editors. *Chemistry and significance of*
 266 *condensed tannins*. New York, N.Y: Plenum Press.
- 267 Clark, A.M. and Hufford, C.D. (1993). *Discco and Development of Novel*
 268 *Prototype Antibiotic for Opportunistic Infections related to the Acquired*
 269 *Immunodeficiency Syndrome*. In human medical agents from plant American
 270 Chemical Society (ACS Symposium Series 534), Washington, DC., pp. 228 –
 271 241.
- 272 Collins, G.H., Lynes, P.M. and Grange, J.M. (1995). *Microbiological Methods*
 273 (7th Edition). Butterworth Heinemann Ltd, Britain. Pp. 175 – 190.

Formatted: German (Germany)

Formatted: German (Germany)

- 274 Davison, H.C., Low, J.C., Woodhouse, M.E. (2000). "What is antibiotic
275 resistance and how can we measure it? *Trend in Microbiology*. 8(112):554 –
276 559. PMD: 11115751.
- 277 Denwick, P.M. (2002). *Natural Products: A Biosynthetic Approach*. 2nd Edition;
278 John Wiley and Sons Ltd., England. Pp. 241 – 243.
- 279 Diallo, D. and Paulsen, B.S. (2000). *Pharmaceutical Research and Traditional*
280 *Practitioners in Mali: Experiences with Benefit sharing. In responding to*
281 *bioprospecting (from biodiversity in the South to medicines in the North).*Edited
282 by: Svarstad H, Dhillion S.S., SpartacusForlagi. pp. 133 – 144.
- 283 Doss, A., Mohammed, H.M. and Dhanabalan, R. (2009).Antibacterial activity
284 of tannins from the leaves of *Solanumtrilobatum* Linn.*Indian J. Sci.*
285 *Technology*.Vol. 2, No. 2. ISSN:0974 – 6848.
- 286 Emeruwa, A.C. (1982). Antibacterial substances from *Carica papaya* fruit
287 extract. *Lioydia*.45(2):123 – 127.
- 288 Fansworth, N.R. (2001). The Present and Future of Pharmacognosy. News
289 Letter of the Americans Society for Pharmacognosy, pp. 4 – 12.
- Enjoka, T. and Kashwada Y. (1996).Anti-AIDS agents. 11. Betulinc acid andplatonc
291 acid as anti-HIV principles form syzigiumClaviflorium and the anti-HIV
292 activity of structurally related triterpenoids. *J. Nat. Prod*. 57:243 – 247.
- 293 Funatogawa, K., Hayashi, S., Shimomura, H., Yoshida, T., Hatano, T., Ito, H.
294 and Iria, Y. (2004). Antibacterial activity of hydrolysable tannins derived from
295 medicinal plants against *Helicobacter pylori*. *Microbiol.Immunol*.48(4):251 –
296 261.
- 297 Gajendiran, N. and Mahadevan, A. (1990).Utilization of Catechin by
298 *Rhizobium* sp. *Plant Soil*. 108, 263 – 266.

- 299 Hemen, D. and Lalilta, L. (2012). A Review on Anthraquinone isolated from
300 Cassie species and their applications.*Indian Journal of Natural Products and*
301 *Resources*. Vol. 3(3):291 – 319.
- 302 Hopp, K.H., Cunningham, L.V.J., Wahba, Khalil, K. (2003). *In vitro*
303 antitrypanosomal activity of certain alkaloids against *Trypanosomalewisi*.
- 304 Jones, G.A., McAllister, T.A., Mur, A.D. and Cheng, K.J. (2006).Effect of
305 Saifonin (*Onobrychisvicitolia*Scop). Condensed tannins on growth and
306 proteolysis by four strain of terminal bacteria. *Appl. Environ. Mmicrobial*.60:
307 1374 – 1378.
- 308 Kurosoki F.and Nishi A (1983) isolation and Antimicrobial activity of
309 the Phytoalexin-6-methoxymellein from cultured carrots cells.
310 *Phytochemistry*. 22(3), 669-672.
- 311 Lin, L.U., Shuwen, L., Shibo, J. and Shuguang, W. (2004). Tannin inhibits
312 HIV-1 entry by targeting gp 41.*ActaPharmacol Sin*. 25(2):213 – 218.
- 313 *Lioydia*.39:375 – 377.(Pub Med).
- 314 Mendez, E., Moreno, A., Colilla, F. Pelaez, F. Limas, G.G., Mendez, R.,
315 Soxiano, F., Salinas, M., DeHaro, C. primary structure and inhibition of
316 protein synthesis in eukaryotic cell fre system of a novel thionin, gamma
317 horothionin from barely endosperm Eur (Biochem, 2000; 194:333339.
318 [pubmed]).
- 319 Morrison, I. M., Asiedu, E. A., Stuchbury, T., and Powell,A. A. (1995),
320 Determination of Liginin and Tannin Contents of Cowpea Seed Coats. *Annals*
321 *of Botany* 76: pp. 287 – 290.
- 322 Nonaka, G.I., Nishioka, I., Nishizawa, M., Yamagishi, T., Kashiwada, Y.,
323 Dutschman, G.E., Bodner, A.J., Kilkuskie, R.E., Cheng, Y.C. and Lee, K.H.

(1990).Anti-AIDS Agents 2. Inhibitory Effect of Tannins on HIV Reverse Transcriptase and HIV replication in H9 lymphocyte cells. *J. Nat. Prod.* 53:387 – 595 (Pub Med).

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)

Oladunmoye, M.K. (2006). Comparative Evaluation of Antimicrobial Activities and Phytochemical Screening of Two of *Acalypha Wilkesiana*. *Trends Applied Sci. Res.* Vo. 1, No. 1, p. 538 – 541.

Pengsuparp, T., Cail, Constant, H., Fong, H.H., Lin, L.Z., Kinghorn, A.D., Ingoltsdottir, K., Wagner, H. (1995). Mechanistic evolution of new plant derived compounds that inhibit HIV-1 reverse transcriptase. *J. Nat. Prod.* 58:1024 – 1031.(Pub Med).

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)

Scalbert, A. (2004). Antimicrobial Properties of Tannin. *Phytochemistry*.30:3875 – 3883.

Schultz, S.C. (1999). Tannin-insect interactions in: Hemingway, R.W., Karchesy, J.J., Editors. Chemistry and Significance of Condensed Tannins. New York N.Y: Plenum Press; 553.

Sofowora, E.A. (1988). Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Limited, Chechester.

Sofowora, E.A. (1993). The State of Medicinal Plants Research in Nigeria, Society of Pharmacology, pp. 54 – 96.

Sofowora, E.A. and Adewunmi, C.O. (1980).Preliminary Screening of some Plant Extracts for Molluscicidal Activity. *Planta Medica*. 39:57 – 65.

Sofowora.A. (1981).Problems and prospects of intergrating Traditional and Western Medicine in Nigeria. *Nigerian Journal of pharmacy*. 12(1):277-283.

- 347 Sowemimo, A.A., Fakoya, F.A., Awopetu, I., Omobuwajo, O.R. and Adesanya,
348 S.A. (2007). Toxicity and Mutagenic Activity of some selected Nigerian Plants.
349 *J. Ethnopharmacol.* 113:427 – 432.
- 350 Steinberg, P.D., Hagerman, A.E., Stern, J.L., Manson, P.K. (1996). Phloro
351 tannin- protein interactions. *J. Chem. Ecol.* 22:1887 – 1899.
- 352 Tijjani, M.A., Abdurahaman, F.I., Abba, A., Idris, Y.S., Baburo, M., Mala,
353 B.S.I., Mala, G.A., Dungus, M.H.M., Aji, B.M. and Abubakar, K.I.
354 (2013). Evaluation of Proximate and Phytochemical Composition of leaves of
355 *Annonasenegalensis*. *Journal of Pharmaceutical & Scientific Innovation*. Vol. 2,
356 Issue 1, p. 7.
- 357 Trease, G.E. and Evans, I.C. (1989). Pharmacognosy (12th Edn) Bailliere Tindall
358 London. pp. 21 – 22.
- 359 Viljoen, A., Van Vuuren, S., Ernest, E., Klepser, M., Demiri, B., Bassar, H. and
360 Van Wyk, B.E. (2003). *Osmitopsis asteriscoides* (Asteraceae)- the antimicrobial
361 and essential oil composition of Cape – Dutch remedy. *J. Ethnopharmacol.* 88,
362 137 – 143.
- 363 Yao, C., Haffney, S.H., Likey, T.H. and Haslam, E. (1999). Carbohydrate –
364 Polyphenol Complex reaction. In Hemingway, R.W., Karchesy, J.J. Editions.
365 Chemistry and Significance of Condensed tannins. New York N.Y: Plenum
366 Press. Pp. 553.

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)

Formatted: Swedish (Sweden)