

Original Research Article**Effect of *Strophanthus hispidus* Dc. (Apocynaceae)****Roots Extract on Normal and Hyperglycemic Rats****ABSTRACT**

Objective: To investigate the hypoglycemic activity of both ethanol extract *Strophanthus hispidus* root (EESHR) and chloroform extract *Strophanthus hispidus* root (CESHR) respectively.

Methods: Both CESHR and EESHR were evaluated respectively for hypoglycemic activity using Fasting blood glucose (FBG) method. CESHR was further fractionated using standard column chromatography techniques and the fractions were evaluated for hypoglycemic using standard oral glucose tolerance test method.

Results: In the experiments, the hypoglycemic activity of the CESHR was better than that of EESHR at 500mg/kg respectively for both extracts which were significant ($p=0.05$) and compared to glibenclamide

CONCLUSION: The results showed that CESHR fraction (3-4) at 200mg/kg possesses significant hypoglycemic activity better than that of the standard drug-glibenclamide. This supports its use as a potent anti-diabetic drug in alternative herbal medicine.

Keywords: *Strophanthus hispidus*, root extract, hypoglycemic, glibenclamide

22 1. INTRODUCTION

23 The oral glucose tolerance tests (OGTT) indicates the body's ability to utilize a type of sugar
24 named glucose that is the body's main source of energy. Oral glucose tolerance tests, a test of
25 immerse value and sentiment, in favour of using fasting plasma glucose concentration alone
26 as a practical attempt to simply the diagnosis of diabetes [1]. Hyperglycemia is an important
27 factor in development and progression of the complication of diabetes mellitus [2].

28 The plant SPH, popularly known as brown *strophanthus*, and hairy *strophanthus* in West
29 Africa, including Nigeria, is indigenous to Africa [3]. A deciduous shrub of 5 m tall and up to
30 100 cm wide, having its stem bark dark grey in colour, with few lenticels, has been reported
31 to have diverse medicinal uses; for example, in the Savannah Zone of West Africa, the latex
32 and seeds of *Strophanthus hispidus* are used as arrow poison, while decoctions of root, stem
33 bark or leaf are used externally to treat skin diseases, leprosy, ulcers, malaria, dysentery,
34 gonorrhea and diabetes [3]. The leaves have been reported to possessed hypoglycemic
35 properties [4]. Similarly, the anti-inflammatory properties of the aqueous of the root extract
36 have been studied [5]. In Nigeria and Ghana, the root decoction is ingested to treat rheumatic
37 diseases and diabetes, while in Togo; the root bark macerate is employed for treating oedema
38 [3]. The trace elements and major elements present in the plant root have been reported by
39 Akinlami et al. [6]. In this study, the blood lowering effects of chloroform and ethanol crude
40 extracts of *S.hispidus* respectively were conducted through oral glucose tolerance test in
41 Wistar albino rats.

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45 2. MATERIALS AND METHODS

46 2.1 Collection of plant materials

47 Fresh roots of *Strophanthus hispidus* were purchased from the market, in Ondo southwest,
48 Nigeria and identified at the Department of Crop, Soil and Pest Management, Federal
49 University of Technology, Akure, Nigeria and specimen sample were kept in the Harbarium.

50 2.2 Extraction

51 The identified plants materials were air dried for four weeks and pulverized into fine powder
52 using clean and sterile Mortar and Blender. The powdered samples were sieved and quartered
53 to obtain a representative of 1000g used for this study. Two hundred grams of the powered
54 roots of *S. hispidus* were extracted with 1L of 96% ethanol or chloroform respectively at
55 room temperature (25-30°C) by maceration for 72h. After the extraction, the solvents were
56 removed from the extracts by distillation and drying at 60°C in a water bath, leaving the
57 extracted portion as a residue. The crude Ethanol Extract *S. hispidus* Root (EESHR) and
58 Chloroform Extract *S. hispidus* Root (CESHR) were stored and kept in air tight bottle in a
59 refrigerator until used for bio-assay. The crude EESHR was dissolved in normal saline to
60 obtain the required concentration of 500mg/kg while CESHR was first dissolved in Tween-80
61 and followed by normal saline to also obtain the needed concentration of 500mg/kg that were
62 required for the bioassay.

63 2.3 Phytochemical screening of crude plant extracts

64 Qualitative phytochemical screening of ethanol extract *S. hispidus* root (EESHR) and
65 chloroform extract *S. hispidus* root (CESHR) respectively were carried-out by standard
66 procedures [7], [8], [9] and [10].

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68 **2.4 Test animals**

69 A sum total of 50 male albino rats weighing about 120-220 gm. of were procured from
70 Animal house of Federal University of Technology, Akure, Nigeria. All the animals were
71 kept in standard polypropylene cages and maintained at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under 12 h. dark/light
72 cycle. The animals were fed with standard rat feed and water was given ad libitum. Ethical
73 clearance for handling the animals was strictly adhered to as described by Akhtar et al. [11].

74 **2.5 Experimental design**

75 Initial screening of the ethanol extract *S. hispidus* root (EESHR) and chloroform extract
76 *S.hispidus* root (CESHR) respectively was done to evaluate their hypoglycemic potential with
77 dosage of 500mg/kg for each of the extracts. These were administered orally by gavage in
78 normal rats by conducting fasting blood glucose (FBG) study. Oral glucose tolerance test
79 (OGTT) studies were done on separated components of chloroform extract *S.hispidus* root
80 (CESHR), which was subjected to Column chromatography. The chloroform extract *S.*
81 *hispidus* root (CESHR) was identified as the most effective hypoglycemic than ethanol
82 extract *S. hispidus* root (EESHR) by initial screening.

83 **2.6 Assessment of hypoglycemic activity in normal healthy rats**

84 The animals were fasted for 18 h with free access to water prior to the administration of the
85 extracts. After fasting the blood glucose levels was measured using the glucose-oxidase
86 principle and the fasting blood glucose greater than 50 mg/dL was included in the study for
87 the normoglycemic group . The normoglycemic rats were randomly assigned into four
88 groups (1-4) of five rats ($n = 5$) each as follows,

89 Group 1: Normal treated with 500 mg/kg of CESHR

90 Group 2: Normal treated with 500mg/kg of EESHR

91 Group 3: Normal treated with 5 ml/kg of normal saline

92 Group 4: Normal treated with 5mg/kg of gibenclamide

93 Fasting blood glucose (FBG) was taken initially and then blood samples were collected from
94 tail vein at 2, 4, 6 and 8 h after administering the extracts.

95 **2.7 Column chromatography separation of chloroform extract *S.hispidus* root (CESHR)**

96 The crude chloroform extract *S.hispidus* root extract was subjected to Column
97 chromatography to further separate the components of the extract into various fractions using
98 the following solvent system: n-hexane, chloroform, ethyl acetate and methanol on silica gel
99 G. Based on their resolutions in Chloroform-EtOAc (5:5), (v/v) on silica gel GF254 thin
100 layer chromatographic plates, the fractions obtained (50ml) were grouped and coded to afford
101 four main fraction: CESHR (3-4), CESHR (5-9), CESHR (10-12), and CESHR (18-25).

102 **2.8 Oral glucose tolerance test in chloroform extract *S.hispidus* root (CESHR)**

103 This test was performed according to [12]. Rats were divided into five groups (n = 6). They
104 were fasted overnight and accessed to water only. Blood was taken from the lateral veins of
105 the tail and the blood sugar levels were initially monitored with a glucometer (One touch
106 Horizon). Group I served as control groups were treated with vehicle (0.5% Tween 80
107 solution), group II treated with 5mg/kg of glibenclamide as a standard drug. Chloroform
108 Fractions CESHR (3-4) serves as group III, CESHR (5-9) served as group IV, CESHR (10-
109 12) served as group V and CESHR (18-25) served as group VI each were treated with a dose
110 of 200 mg/kg and After 30 min, the animals were treated with 5% (wt/v) glucose orally.
111 Blood glucose levels were monitored from lateral tail veins at 30, 90 and 150 min intervals
112 after post glucose challenge. Data were expressed as mean $\pm$ standard error of mean.
113 Statistical comparison were performed by one-way ANOVA followed by Dunnett's multiple
114 comparison test, and the values were considered statistically significant when $p = .05$.

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3. RESULT AND DISCUSSION

3.1 Qualitative Phytochemical Screening

Tables 1. presents the result of Qualitative phytochemical screening of ethanol extract *S. hispidus* root (EESHR) and chloroform extract *S. hispidus* root (CESHR) respectively. Both extracts contained flavonoids, alkaloids, saponins. Cardiac glycosides, tannins and anthraquinones. The results are in agreement with similar research work carried out by Agbaje and Fageyinbo [13]. Phytochemicals compounds present as secondary metabolites play vital roles in the bioactivity exhibited by medicinal plants used ethno botanically [14]. Some plant compounds have pharmacology effects, which forms the basis of chemical synthesis of drugs used in modern medicine [14]. Flavonoids are polyphenols compounds that are structurally classified as flavonols, flavones, isoflavones, catechins, anthocyanidins and chalcones [16]. Numerous flavonoids have been identified; many of which occur in fruits, vegetables and beverages tea, coffee, beer, wine and fruit drinks [17]. Flavonoids (Figure 1) have potential beneficial effects on human health. They have been reported to have anti-viral, anti-platelet, anti-inflammatory, anti-tumors, anti-diabetic and antioxidant activities [18]; [16].

Table 1: Qualitative phytochemical screening of ethanol extract *S. hispidus* root (EESHR) and chloroform extract *S. hispidus* root (CESHR) respectively.

General Test	Specific Test	EESHR	CESHR
Test for Flavonoids	Ferric Chloride Test	+	+
Test for Glycosides	Keller-Kiliani Test	+	+
Test for Tannins	Ferric Chloride Test	+	+
Test for Alkaloids	Dragendoffs test	+	+
Test for Anthraquinones	Hydrogen Chloride Test	+	+

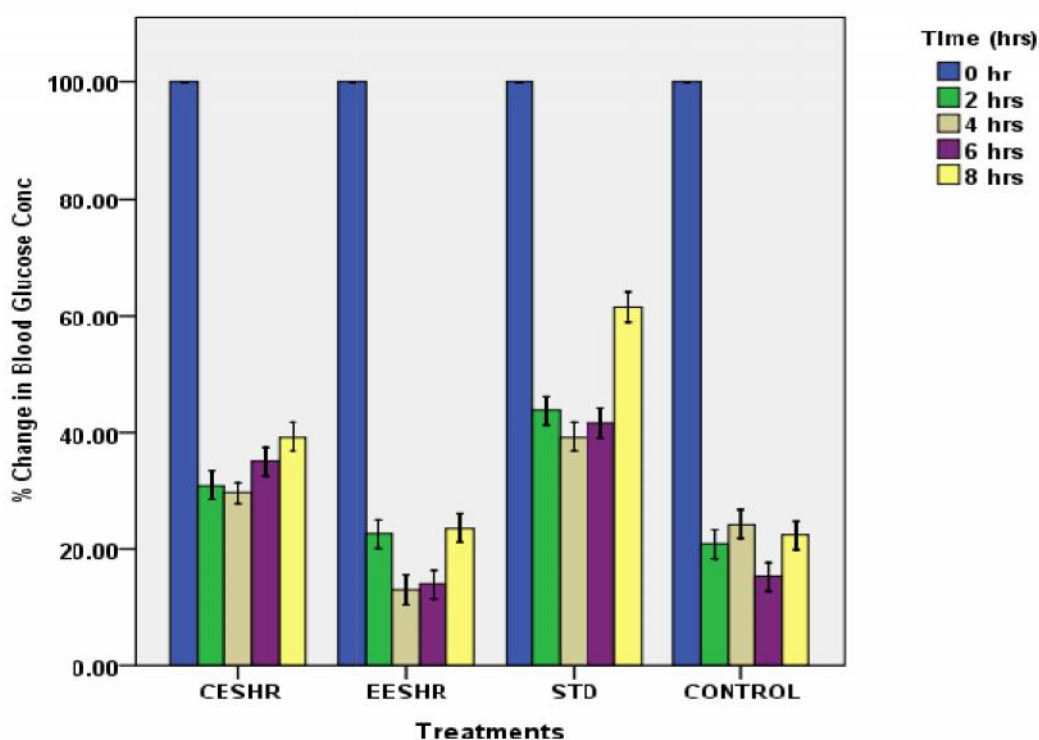
144	Test for Saponins	Frothing Test	+	+
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146 3.2 Hypoglycemic effect of extracts on normal rats

147 Figure 1 describes the result of hypoglycemic effect of chloroform extract of *S.hispidus* roots
 148 (CESHR) and ethanol extract of *S.hispidus* roots (EESHR) respectively, on fasting blood
 149 glucose (FBG) of normal rats. Rats treated with 500mg/kg CESHR showed a maximum fall
 150 of 39.2% in Fasting blood glucose (FBG) after 8hrs. of oral administration, while the fall of
 151 26.62% was observed with 500mg/kg of EESHR respectively. The hypoglycemic activity of
 152 the chloroform root extract was better than that of ethanol root extract. The result was in
 153 agreement with similar report by of Ojiako et al. [19].

154 **Fig.1. Hypoglycemic effect of *S.hispidus* root extracts on BGL of normal rats**



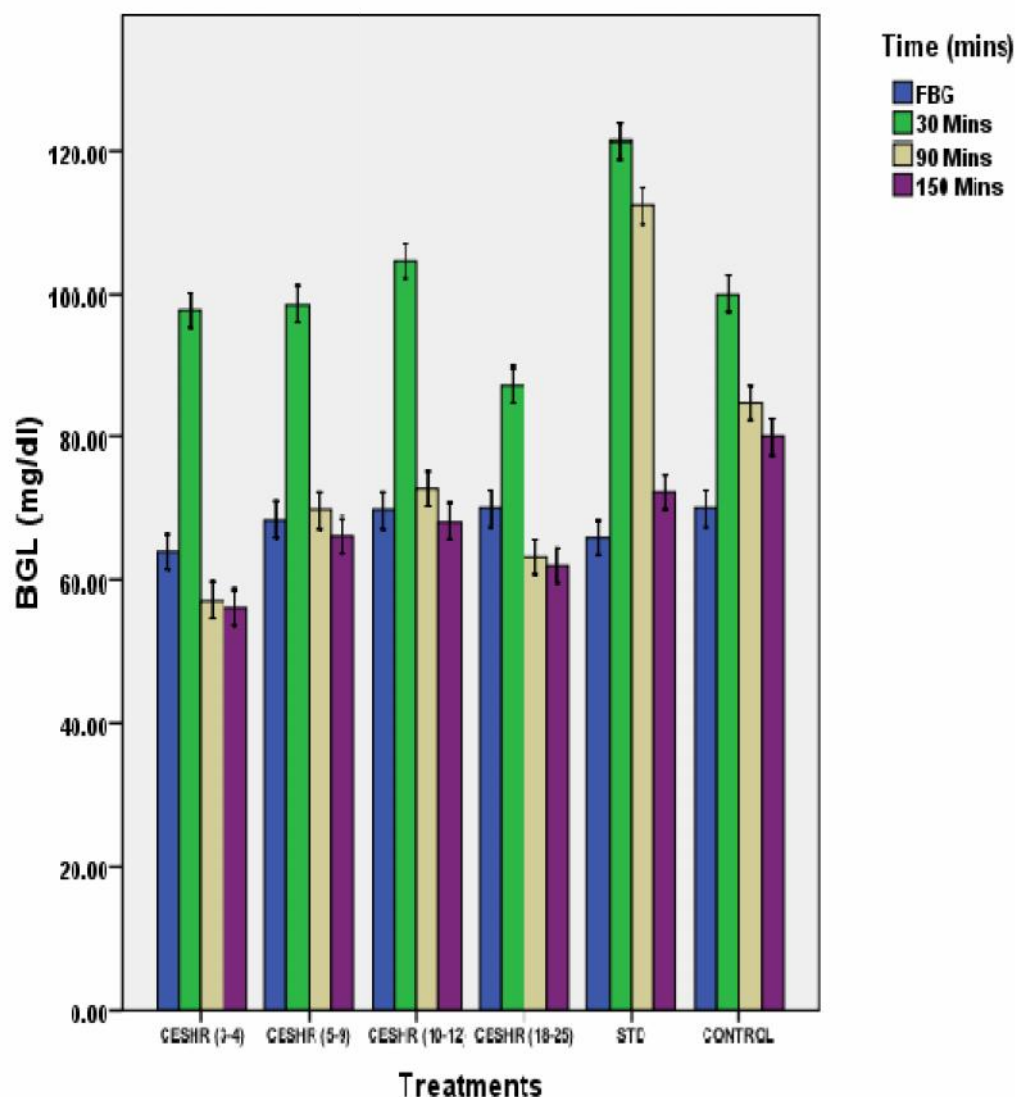
155 Each value shown in mean $\pm$ S.D. (n = 5). Control (distilled water); Chloroform extract
 156 *S.hispidus* roots (CESHR-500mg/kg); Ethanol extract *S.hispidus* roots (EESHR-500mg/kg);
 157 STD (gibenclamide -5mg/kg).
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3.3 Hypoglycemic effect of chloroform extracts fractions on OGTT of normal rats

The chloroform crude extract was subjected to Column chromatography to further separate the components of the extract into various fractions using the following solvent system: n-hexane, chloroform, ethyl acetate and methanol on silica gel G. Based on their resolutions in Chloroform-EtOAc (5:5), (v/v) on silica gel GF254 thin layer chromatographic plates, the fractions obtained were grouped and coded CESH (3-4), CESH (5-9), CESH (10-12), and CESH (18-25). Figure 2 depicts the hypoglycemic effect of a single oral administration of 200mg/kg each of different chloroform extract fractions of *S.hispidus* root on OGTT of normal rats. Experimental induction of hyperglycemia by intragastric ingestion of glucose resulted in a 1.5 to 2-fold increase in plasma glucose levels (comparing bar groups of 0 minute with the bar groups of 30 minute, (fig. 2). The CESH fraction (3-4) produced a maximum fall of 42.45% at two hours after glucose administration, whereas fall of 32.74%, 34.66% and 28.93% were observed with the CESH fraction (5-9), CESH fraction (10-12) and CESH fraction (18-25) respectively. A standard antihyperglycemic drug, glibenclamide, when administered to glucose loaded rats, reduced blood glucose level by 49.00%. The results demonstrated that the CESH fraction (3-4) possesses antihyperglycemic potential more than the others fractions. The CESH fraction (3-4) was comparable to that of the standard drug, glibenclamide, in its effectiveness in lowering blood glucose. This suggests that the fraction contains antihyperglycemic constituents.

195 **Fig.2. Hypoglycemic effect of Chloroform extract fractions *S.hispidus* roots (CESHR)**
 196 **on BGL of normal rats during OGTT**



197 .Each value shown in mean $\pm$ S.D. (n = 6). Control ((0.5% Tween 80 solution); Chloroform
 198 extract fractions *S.hispidus* roots (CESHR-200mg/kg); STD (glibenclamide-5mg/kg).
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200
 201 However, it can be suggested that the mode of action of action of fractions CESHR (3-4) is
 202 probably mediated by an enhanced secretion of insulin, like glibenclamide a biguanides. In
 203 the light of the above pharmacology results, further works is on-going in our laboratory to
 204 possible isolate and charaterize the possible hypoglycemic compounds from the most active
 205 fraction using Infra-red spectroscopic, ^1H , ^{13}C , 2D-NMR spectroscopic and Electron Spray

206 Mass spectroscopy. Finally, the present study has given idea of the hypoglycemic compounds
207 present in the reported plant fraction.

208 **4. CONCLUSION**

209 The result showed that CESH fraction (3-4) at 200mg/kg possesses significant
210 hypoglycemic activity better than that of the standard drug-glibenclamide. This supports its
211 use as a potent anti-diabetic drug in alternative herbal medicine.

212 **CONSENT**

213 It is not applicable

214 **ETHICAL APPROVAL**

215 The authors declare that this work was not against public interest. Animal experiments were
216 conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub.
217 No. 85-23, Revised).

218 **REFERENCES**

- 219 1. Famobuwa OE, Akinlami OO, Agbowuro AA. Oral glucose tolerance test in normal
220 control and glucose induced hyperglycemic rats with *Tetracarpidium conophorum*
221 (Nigerian Walnuts). Journal of Natural Products and Biomedical Research.
222 2015;1(2),48-50.
- 223 2. Luzi I. Pancreas transplantation and diabetic complications. The New England Journal
224 of Medicine. 1998; 339, 115-117.
- 225 3. Hutchinson J and Dalziel JM. Flora of West Tropical Africa. II. Millbank, London
226 crown agents for oversea Government and Administration 4, 1963; 221-221.

- 227 4. Ojiako, OA. and Igwe, CU. A time-trend hypoglycemic study of ethanol and
 228 chloroform extracts of *Strophanthus hispidus*. Journal of Herbs, Spices and Medicinal
 229 Plants. 2009; 15(1), 1-8.
- 230 5. Agbaje, EO and Fageyinbo, MS. Evaluating Anti-Inflammatory activity of aqueous
 231 root extract of *Strophanthus hispidus* DC. (Apocynaceae) International Journal of
 232 Applied Research in Natural Products. 2012; 4 (4), 7-14.
- 233 6. Akinlami, O., Lajide, L., Oloyede, H and Ojo, B. Trace Elements and Majors
 234 Minerals Evaluation in *Fluerya aestuans* Linn. (Urticaceae). International Journal
 235 Pharma Science. 2014; 3(5): 333-337.
- 236 7. Harborne, JB. Methods of plant analysis. In: Phytochemical Methods. Chapman and
 237 Hall, London; 1980, 67-78.
- 238 8. Sofowora, LA. African Medicinal plants proceeding of conference, university of Ife,
 239 Nigeria. 1982; 70-73.
- 240 9. Edeoga, HO., Okwu, DE, and Mbaebie, BO. 2005. Phytochemical constituents of
 241 some Nigerian medicinal plants. Africa journal of Biotechnology. 2005; 4(7), 685-
 242 688.
- 243 10. Nayak, BS and Pinto, LM. *Catharanthus roseus* flower extract has wound-healing
 244 activity on Sptague Dawley rats. BMC Complementary and Alternative Medicine
 245 2006; 6, 41.
- 246 11. Akhtar, MA, Rashid, M, Ibne Waheed, MI, Islam, MR, Shaheen, SM, Islam, MA and
 247 Ahmed, M. Comparison of large-term antihyperglycemic and hypolipidemic effects
 248 between *Coccinia cordifolia* (Linn) and *Catharanthus roseus* (Linn) in alloxan-
 249 induced diabetic rats. Research of Medical Sciences Medical Science. 2007; 2(1), 29-
 250 34.

- 251 12. Shirwaikar, A and Rajendran, K. Effect of aqueous bark extract of *Garuga pinnata*
252 *Roxb.* in streptozotocin-nicotinamide induced type-II diabetes mellitus. *Journal of*
253 *Ethanopharmacology*.2006; 112, 1-6
- 254 13. Agbaje, EO and Fageyinbo, MS. Evaluating Anti-Inflammatory activity of aqueous
255 root extract of *Strophanthus hispidus* DC. (Apocynaceae). *International Journal of*
256 *Applied Research in Natural Products*. 2012; 4 (4), 7-14.
- 257 14. Kar, A and Choufhary, BK. Important mineral content of a few Ayurvedic herbs with
258 a discussion on medicinal aspects. *Indian Drugs*.1994; 31(3): 127-130
- 259 15. Lee, KV, KIM, YJ, Lee, HJ and Lee, CY. Cocoa has more Phenolic Phytochemical
260 and higher antioxidant capacity than teas and red wine. *Journal of Agriculture and*
261 *Food Chemistry*. 2003; 51(25):7292-5
- 262 16. Wikipedia., Flavonoids.en.wikipediaedi.org/wiki/Flavonoids, 2012b.
- 263 17. Rice-Evans, C.A. Flavonoids and isoflavonones absorption, metabolism and
264 bioactivity. *Free Radical and Biological Medicine*. 2004; 36:827-828.
- 265 18. Ojiako, OA and Igwe, CU. A Time-Trend Hypoglycemic Study of Ethanol and
266 Chloroform Extracts of *Strophanthus hispidus*. *Journal of Herbs, Spices & Medicinal*
267 *Plants*. 2009; 4, 45-49.