

Original Research Article

PHYTOCHEMICAL SCREENING AND COMPARATIVE ANTIOXIDANT ACTIVITIES OF DIFFERENT FRACTIONS OF *SONNERATIA* *CASEOLARIS* (LINN.) BARKS EXTRACTS.

ABSTRACT

Aims: Our study was carried out to appraise the phytochemical screening and antioxidant potentials of different fractions of *Sonneratia caseolaris* (Linn.) barks extracts.

Study design: For the purpose of this experiment the extracts were subjected for an *in-vitro* study.

Place and Duration of Study: The study was carried out in August 2014 in the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

METHODOLOGY : The various fractions of *Sonneratia caseolaris* (Linn.) barks extracts as Ethanolic fractions (ETF), ethyl acetate (EAF), chloroform (CLF) and pet ether (PTF) fractions obtained after extraction were subjected to preliminary phytochemical screening. The antioxidant capacity of Ethanolic fractions (ETF), ethyl acetate (EAF), chloroform (CLF) and pet ether (PTF) extracts of barks of *S. caseolaris* were evaluated using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay. Total antioxidant activity and Total phenolic content of Ethanolic fractions (ETF), ethyl acetate

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Comment [U2]: do not write repeated ethanol fractions (ETFs), ethyl acetate (EAF), chloroform (CLF) and pet ether (PTF) for the second time, henceforth enough abbreviations only

(EAF), chloroform (CLF) and pet ether (PTF) extracts of *S. caseolaris* were determined.

RESULTS: The phytochemical screening showed the presence of Flavonoid, Steroid, Tannin compounds in large amount. In DPPH scavenging assay among the extracts, Ethanolic fractions (ETF) exhibited the highest DPPH scavenging activity with IC_{50} of 4.57 μ g/ml. The highest phenolic content was found in EAF extracts (63.00 mg of GAE / gm. of dried extract) followed by CLF (36.25 mg of GAE / gm. of dried extract) and PTF (26.28 mg of GAE / gm. of dried extract). The highest total antioxidant activity was also found in ETF fraction (185 GAE/gm of dried sample followed by EAF fraction (99.00GAE/gm of dried sample), PTF (84.00 GAE/gm of dried sample) and Chloroform (49.00 GAE/gm of dried sample).

CONCLUSION: Our result demonstrates that all the extractives of *S. caseolaris* have appreciable antioxidant activities.

Comment [U3]: 185.00 ? two decimals consistent with the other

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KEYWORDS: *Sonneratia caseolaris* ,DPPH, Total antioxidant activity, Total phenolic content.

Comment [U4]: Keywords are usually arranged in alphabetical

16 **1. INTRODUCTION**

17

18 *Sonneratia caseolaris* (L.) (Sonneratiaceae) is such a mangrove plant found widespread in
19 tropical and subtropical tideland. *S. caseolaris* is a medium-size plant (2-20m high),
20 evergreen tree with elliptic-oblong leaves (5-9.5cm long) [1-2]. *S. caseolaris* is reported to
21 have 24 compounds such as nine triterpenoids, eight steroids, three flavonoids and four
22 benzene carboxylic derivatives have been isolated from stems and twigs of medicinal
23 mangrove plant *S. caseolaris* [3]. This plant contains phenolic compound like gallic acid and
24 flavonoids e.g. luteolin and luteolin-7-O-glucoside [4]. It contains alkaloid, tanin, flavonoid,
25 saponin, phytosterol, and carbohydrate[5-6].*S. caseolaris* to be used in traditional medicine
26 systems in several countries, it is used for sprains, swelling helminthiasis, poultices, coughs,
27 hematuria, small pox, astringent, antiseptic, arresting hemorrhage, piles, and also used as
28 remedy to stop blood bleeding [7]. *S. caseolaris* possessed intestinal α -glucosidase
29 inhibitory property [8] and it has also been reported to be toxic against mosquito larvae [7].

UNDER PEER REVIEW

30 Oxidative stress follow-on due to imbalance of oxidizing agents and natural antioxidants in
31 the body induces the brutality of a number of diseases like atherosclerosis, cancer,
32 cardiovascular ailments, neurodegenerative disorders and diabetes [9]. As self-protective
33 measure against such oxidative damages, biological systems have evolved a range of
34 enzymatic machineries and scavengers. These include dietary antioxidants (α-tocopherol, β-
35 carotene, ascorbic acid, glutathione, uric acid), hormones (estrogen, angiotensin), enzyme
36 systems (superoxide dismutase, glutathione peroxidase, catalase) etc. [10-11]. A large
37 number of antioxidative agents, both natural (e.g. α-tocopherol) and synthetic (e.g. butylated
38 hydroxyanisole, butylated hydroxytoluene, tert-butyl hydroquinone and propyl gallate) are
39 broadly used in the food industry to lengthen shelf life as they inhibit lipid oxidation [12].
40 However, the use of these synthetic antioxidants is increasingly getting restricted because of
41 their toxicity and health risks [13]. Therefore, discovery of novel antioxidative of natural origin
42 is the urgent need of the hour and plants can be a good source for the purpose [12]. Earlier
43 research focused on methanolic bark extracts to illustrate the antioxidant activity of *S.*
44 *caseolaris*. However, here we focus on comparative antioxidant activities of different
45 fractions of *Sonneratia caseolaris* (Linn.) barks extracts.

46

47 2. METHODS

48 2.1 Collection, identification and preparation of plant material

49 The stems were harvested after identification by an expert taxonomist from the plant growing
50 at Barisal on August 5, 2014. The stems were dried under shade at room temperature for a
51 period of two weeks in order to avoid solar radiations from altering the **API**. These stems
52 were spread on plastic bags while avoiding their stacking. Every day we turned these stems
53 upside down so that to favor a homogenous drying process. The dried leaves were ground in
54 a clean electric grinding machine in such a way to obtain a **fined powder**, which was stored
55 in an airtight container. The total dried powder material was obtained 600 gm. It was divided
56 equally into four portions and was refluxed with ethanol, ethyl acetate, pet ether and

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Comment [U6]: how much size is the mesh?

57 chloroform solvent for three times The extract was filtered with **Whiteman** No. 1. filtered
58 paper and the collected filtrate was evaporated in an oven at 50°C. This extract was weighed
59 so that to determine the yield obtained from the initial powder quantity and then stored in an
60 air-tight container for subsequent experimental tests.

Comment [U7]: Whatman ?

61 **2.2.1 Phytochemical screening**

62 Phytochemical screening of the stems extracts of *S.caseolaris* were tested for the presence
63 of active principles such as alkaloids, flavonoids, tannins, reducing sugar etc. using the
64 standard procedures.

65 Test for saponin:

66 About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and
67 filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a
68 stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken
69 vigorously, then observed for the formation of emulsion.

70 Test for saponins (Kokate, 1999): The extract was diluted with distilled water and made up
71 to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam
72 indicates the presence of saponins.

73 Test for Tannins: About 2.5 g of the plant extract was dissolved in 5 ml of distilled water,
74 filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green
75 precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1989).

76 Test for Flavonoid

77 The presence of flavonoids in the samples was determined using the methods [14]. 10 ml
78 Ethyl acetate was added to 0.2g of the powdered sample and heated in a water bath for 5
79 min. The mixture was cooled , filtered and the filtrates used for the test. **Ammonium test:**
80 About 4 ml filtrate is shaken with 1 ml of dilute ammonia solution. The layer is allowed to
81 separate and the yellow color in the ammoniacal layer indicates the presence of
82 flavonoids. Aluminum chloride solution test: 1 ml of 1% aluminum chloride solution is added
83 to 4 ml of the filtrate and shaken. A yellow coloration indicates the presence of flavonoids.

84 Test for alkaloids

85 Mayer's test (Evans, 1997): To a few ml of the filtrates, a drop of Mayer's reagent was added
86 by the side of the test tube. A creamy or white precipitate indicates the test is positive.

87 Test for carbohydrates

88 Benedict's test: To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture
89 was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates
90 the presence of sugar. [15]

Comment [U8]: Too small volume, should be 5 ml of Benedict' reagent

91 Test for steroids

92 Two ml of acetic anhydride was added to 0.5 g of extracts of each sample with 2 ml H₂SO₄.

93 The colour changed from violet to blue or green in some samples indicating the presence of
94 steroids. [15]

95 **2.2 Evaluation of Antioxidant activity**

96 **2.2.1 Estimation of Total Phenolic content**

97 The Folin-Ciocalteu's reagent was used as oxidizing agent and test-using gallic acid as
98 standard, the total phenolic content of extractives of *S.caseolaris* was described by Singleton
99 *et al* [16] with some modifications. The assay mixture consisted of extract (0.5 ml that was
100 adjusted to 1.0 ml with distilled water) and 2.5 ml of Folin-Ciocalteu's reagent. Furthermore,
101 after incubation at room temperature for 15 min, 2.5 ml of (w/w) Na₂CO₃ solution was added
102 into the test tube and the test tube was incubated at the same temperature for 20 minutes.
103 Finally, the absorbance was read at 760 nm against reagent blank. However, the methanol
104 extract and in different fractionates in Gallic acid equivalents (GAE) was calculated by the
105 according to the formula.

$$106 \quad C = (c \times V)/m$$

107 Where,

108 C = total content of phenolic compounds, mg/g plant extract, in GAE; c= the concentration of
109 Gallic acid established from the calibration curve, mg/ml; V = the volume of extract, ml; m =
110 the weight of different pure plant extracts, gm.

111 **2.2.2 Estimation of Total antioxidant capacity**

112 Catechin reagent was used as a standard; the total antioxidant capacity of extractives of *S.*
113 *caseolaris* was determined by the method of Prieto *et al* [17].with slight modifications. The
114 experiment mixture consisted of extracts (0.5 ml standard or plant extract solution) was
115 taken in a test tube with 3 ml of reaction mixture containing 0.6 M sulphuric acid, 28 mM
116 sodium phosphate and 1% ammonium molybdate was added into the test tube. In addition,
117 after incubation at 95°C for 10 minutes, the absorbance of the solution was read at 695 nm
118 against reagent bank using a spectrophotometer. The experiment was done three times at
119 each concentration.

120 **2.2.3 DPPH Radical scavenging Assay**

121 The 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was evaluated
122 with the help of the method used by Fresin [18]. using modified procedure. Test samples
123 were prepared by dissolving 5 mg of dry extracts in 5 ml of methanol. The assay mixture
124 contained extract (0.5 ml) and DPPH (1.0 ml) which were mixed well and incubated in the
125 dark for 30 minutes. The blank was prepared and made to contain methanol (0.5 ml) and
126 DPPH (1.0 ml). The absorbance was measured at 517 nm on a visible spectrophotometer.
127 All experiments were performed in triplicate. DPPH radical activity was calculated by the
128 following equation.

129
$$\% I = \{(A_0 - A_1)/A_0\} \times 100$$

130 Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.
131 In addition, % inhibitions were plotted against concentration and from the graph IC_{50} was
132 calculated.

133 **3. RESULTS**

134 **Table 1. Phytochemical test results of different extractives of *S. caseolaris***

135

Phytochemical tests	Crude methanol extract	Ethanol fraction	Chloroform fraction	Petroleum Ether fraction	Ethyl acetate fraction
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Comment [U9]: You should move the result to the next page so that the table is not interrupted.

Saponin	++	+	-	-	+
Tannin	+++	+++	++	++	++
Flavonoid	+++	+++	++	++	++
Alkaloid	++	+	-	-	+
Carbohydrate	++	++	-	+	-
Steroid	+++	++	++	+	+

136 Here, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large
 137 amount, - = Not
 138 present

139 3.1 Determination of Total Phenolics

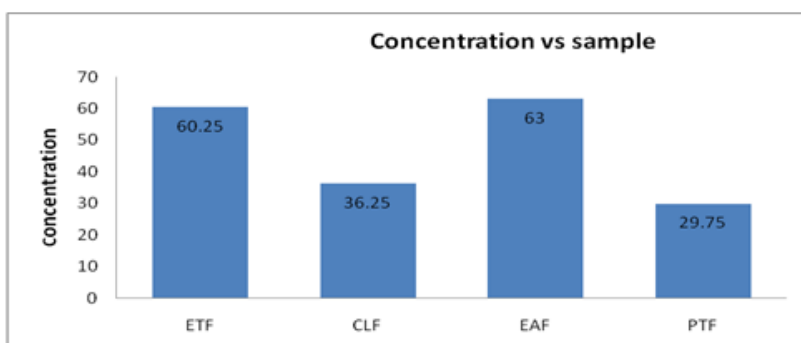
140 The results were expressed as mg of Gallic Acid Equivalent (GAE)/gm of dried extractives.
 141 Among the fractions the highest phenolic content was found in EAO fractions (63.00 mg of
 142 GAE / gm of dried extract) followed by ETF (60.25 mg of GAE / gm of dried extract), CLF
 143 (36.25 mg of GAE / gm of dried extract) and PET (26.28 mg of GAE / gm of dried extract).
 144 Comparing the phenolic content of different fractions of *S. caseolaris* it was observed that
 145 EAO contains considerable amount of phenolic compounds than the other extracts. However,
 146 phenolic content of the samples were calculated on the basis of the standard curve for gallic
 147 acid as shown in table 2 and in figure 01.

Comment [U10]: Tabel , Figure, capitalized each word.

148 **Table 2: Determination of total phenolic content of different fractions of *S.***
 149 ***caseolaris*.**

Sample	Conc. (µg/ml)	Absorbance	GAE/gm of dried sample
Ethanol fraction	250	0.296	60.25
Chloroform fraction	250	0.25	36.25
Ethylacetate fraction	250	0.324	63.00
Pet-ether fraction	250	0.174	26.28

151



152

153 **Figure 01: Total phenolic content of different fractions of barks of *S. caseolaris*.**

154 Here, ETF = Ethanol fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction,
 155 PTF = pet-ether fraction.

156 **3.2 Determination of total antioxidant activity**

157 Total antioxidant activity of four different solvents of crude extract such as ethanol (ETF),
 158 chloroform (CLF), ethyl acetate (EAF) and pet-ether fraction (PTE) were investigated.
 159 Among the four different fractions ETF showed the highest total antioxidant activity with
 160 absorbance at 200 µg/ml concentration followed by EAF (absorbance of 0.388 at 200
 161 µg/ml), PTF (absorbance of 0.187 at 200 µg/ml) and CLF (absorbance of 0.166 at 200
 162 µg/ml). Our result demonstrates that all the extractives of *S. caseolaris* have appreciable
 163 total antioxidant activity. However, total antioxidant activity of plant extracts and (+)-catechin
 164 (standard) were depicted in table 03 and 04 and in figure 02 and 03.

165 **Table 03: Absorbance of catechin (standard) at different concentrations for**
 166 **determination of total antioxidant activity.**

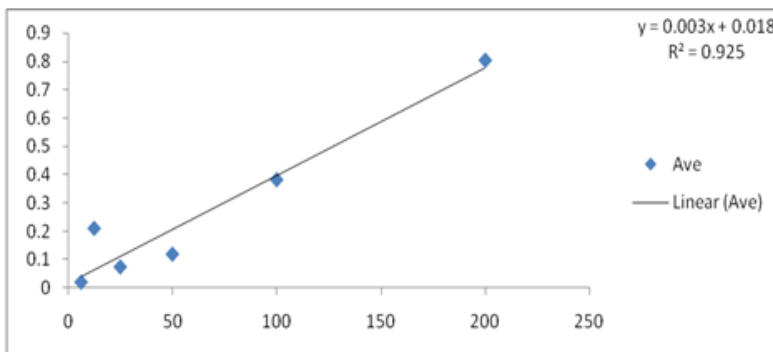
Comment [U11]: I recommend that Table 3 move to the next page so that it is not interrupted

167

Name of sample	Concentration (µg/ml)	Absorbance			Absorbance Mean ±STD
		a	b	c	
	6.25	0.018	0.019	0.021	0.019± 0.0015
	12.5	0.207	0.211	0.209	0.209 ± 0.002

(+) - Catechin	25	0.037	0.039	0.035	0.037 ± 0.002
	50	0.118	0.119	0.116	0.117 ± 0.001
	100	0.380	0.383	0.379	0.381 ± 0.002
	200	0.803	0.801	0.805	0.803 ± 0.002

168



169

170 **Figure 02: Standard curve of catechin for the determination of total antioxidant**
 171 **capacity.**

172

173 **Table 04: Determination of total antioxidant capacity of different solvent fractions**
 174 **of crude ethanolic extract of *S. caseolaris*.**

Sample	Conc. (µg/ml)	Absorbance	GAE/gm of dried sample
Ethanol fraction	200	0.388	185
Chloroform fraction	200	0.166	49.00
Ethyl acetate fraction	200	0.216	99.00
Pet-ether fraction	200	0.187	84.00

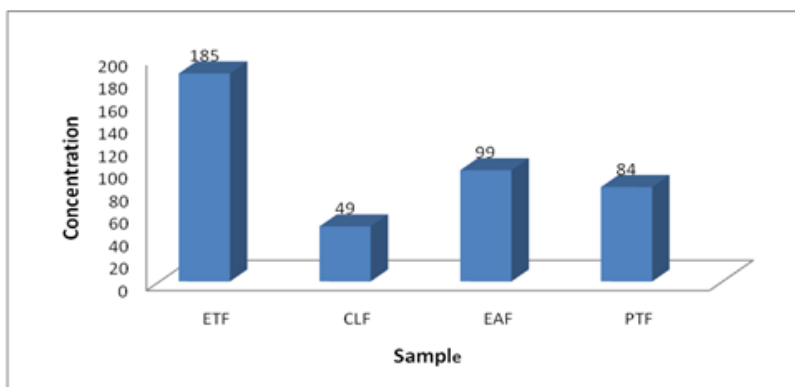
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180
181 **Figure 03: Total antioxidant activity of different solvents fractions of the extracts of *S.***
182 ***caseolaris*.**

183
184 **3.3 Determination of DPPH radical scavenging activity**

185 DPPH radical scavenging activity of different fractions of solvents of ethanolic (ETF),
186 chloroform (CLF), ethyl acetate (EAF) and pet-ether fraction (PTE) were investigated.
187 Among all extracts ethanol fraction (EAF) showed the highest DPPH radical scavenging
188 activity with IC_{50} value of 4.57 $\mu\text{g/ml}$ and chloroform fraction ethanol fraction (EAF) showed
189 the lowest DPPH radical scavenging activity with IC_{50} value of 197.27 $\mu\text{g/ml}$ respectively. The
190 results of DPPH radical scavenging assays of plant extracts and butylated hydroxytoluene
191 (BHT) (standard) are given in table 05 and in figure 04.

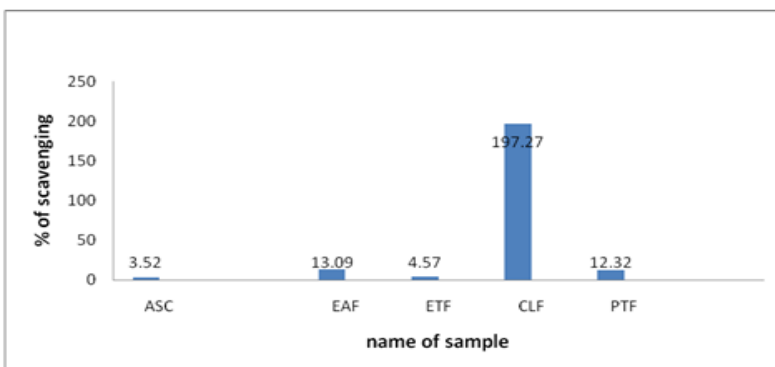
192 **Table 05: DPPH radical scavenging activity of different fractions of extracts of *S.***
193 ***caseolaris* and BHT (Standard) at different concentrations.**

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Name of sample	Conc. ($\mu\text{g/ml}$)	Absorbance	% of scavenging	IC_{50} ($\mu\text{g/ml}$)
BHT	200	0.073	94.45	3.25 $\mu\text{g/ml}$
	100	0.071	94.48	
	50	0.079	94.33	
	25	0.085	93.40	
	12.5	0.098	92.39	

194

	6.25	0.147	88.58	
Ethanol fraction	200	0.085	93.40	4.57 µg/ml
	100	0.073	94.33	
	50	0.071	94.48	
	25	0.080	93.78	
	12.5	0.126	90.21	
	6.25	0.409	68.24	
Chloroform fraction	200	0.635	50.69	197.27 µg/ml
	100	1.038	19.40	
	50	0.675	47.59	
	25	0.707	45.10	
	12.5	0.935	27.40	
	6.25	0.689	46.50	
Ethyl acetate fraction	200	0.061	95.26	13.09 µg/ml
	100	0.228	82.29	
	50	0.432	66.45	
	25	0.555	56.90	
	12.5	0.673	47.74	
	6.25	0.697	45.85	
Pet-ether fraction	200	0.749	41.84	12.32 µg/ml
	100	0.637	51.47	
	50	0.698	45.80	
	25	0.742	42.39	
	12.5	0.635	50.69	
	6.25	0.524	59.31	



196
197 **Figure 04: IC₅₀ (µg/ml) values of different extractives of *S. caseolaris* for DPPH radical**
198 **scavenging activity.**

199
200 **4. DISCUSSION**

201 At maximum wavelength at 517 nm, The DPPH free radical can easily accept an electron or
202 hydrogen from antioxidant molecules to develop into a stable diamagnetic molecule .Due to
203 the DPPH radical's ability to bind hydrogen, it is considered to have a radical scavenging
204 property. Discoloration occurs due to the decreasing quantity of DPPH radicals in the
205 environment. The discoloration of the DPPH therefore reflects the radical scavenging activity
206 of the analyzed extracts [19].Based on the data obtained from this study, DPPH radical
207 scavenging activity of *S. caseolaris* extract of chloroform fraction (IC₅₀ 4.57µg/ml) was
208 similar to that standard BHT ((IC₅₀ 3.25 µg/ml).

209 Phenolic compounds have redox properties, which let them to act as antioxidants. [19]. Free
210 radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic
211 concentration could be used as a basis for rapid screening of antioxidant activity. Among the
212 fractions the highest phenolic content was found in EAF(63 mg of GAE / gm. of dried extract)
213 and then ETF (60.25 of GAE / gm. of dried extract) ,CLF(36.25 mg of GAE / gm. of dried
214 extract) and PTF(29.75 mg of GAE / gm. of dried extract) . Comparing the phenolic content
215 of ETF, EAF, CLF and PTF extracts of *S. caseolaris* it was observed that ETF contains
216 considerable amount of phenolic compounds than the other extracts.

217 The total antioxidant capacity (TAC) was based on the reduction of Mo(VI) to Mo(V) by the
218 extract and subsequent formation of greenphosphate/Mo(V)complex at acid pH . It evaluates
219 both water-soluble and fat-soluble antioxidants. Among the different extracts, Ethanol
220 fraction showed the highest total antioxidant activity (185 GAE/gm of dried sample).

221 5. CONCLUSION

222 Our study investigation brings out the scientific rationale for the folkloric uses of the plant in
223 the management of oxidative stress associated disorders. Even so, further research is
224 needed towards isolation and ascertainment of active principles present in the extracts,
225 which could possibly be explored for pharmaceutical use.

226

227 COMPETING INTERESTS

228 There are no competing interests.

229 CONSENT: NOT APPLICABLE

230 ETHICAL APPROVAL: NOT APPLICABLE

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Comment [U13]: there are 12 from 19 references out of date (over ten years)

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