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Oral Antidiabetic Activities of Different Extracts of *Caesalpinia bonducella* Seed Kernels

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Abstract

Caesalpinia bonducella F. (Leguminosae) is a medicinal plant, widely distributed throughout India and the tropical regions of the world. Its seed kernels are used in the management of diabetes mellitus, in the folklore medicine of Andaman and Nicobar as well as the Caribbean Islands. The seed kernel powder was reported to have hypoglycaemic activity in experimental animals. Four extracts (petroleum ether, ether, ethyl acetate and aqueous) of the seed kernels were prepared and tested for their hypoglycaemic potentials in normal as well as alloxan induced diabetic rats. In normal rats, only ethyl acetate and aqueous extracts showed a minimum significant hypoglycaemic effect, compared to that of glibenclamide. In diabetic rats, both the polar extracts (ethyl acetate and aqueous) as well as glibenclamide, showed significant hypoglycaemic effect, besides, reversing the diabetes induced changes in lipid and liver glycogen levels. As far as the non-polar extracts were concerned, the ether extract showed a marginal antidiabetic activity, while the petroleum ether extract failed to show any. Since both the polar extracts were, chemically, found to contain triterpenoidal glycosides, we presume that they might be the active principles contributing to the antidiabetic actions. In *in vitro* antioxidant studies, the aqueous extract was found to be devoid of any free radical scavenging activity, while the ethyl acetate extract showed a maximum of 49% activity at the end of 1 h. Although the antioxidant potential of ethyl acetate extract may contribute to overcome the diabetes linked oxidative stress, it needs not necessarily contribute to its hypoglycaemic activity.

Keywords: Alloxan-induced diabetes, antidiabetic, antioxidants, *Caesalpinia bonducella* F., hypoglycaemic effect, seed kernels.

Latin binomial: *Caesalpinia bonducella* Fleming.

Introduction

Caesalpinia bonducella F. (syn. *Caesalpinia crista*) (Leguminosae), popularly known as "Gajagakayi" in Kannada and "Fever nut" in English, is a medicinal plant, widely distributed throughout India and the tropical regions of the world. Each and every part of the plant is claimed to possess some therapeutic property, but the seed kernel is the most widely used part, all over the world, in various systems of medicine. It is reported to have antipyretic, antidiuretic, antibacterial, anthelmintic (Neogi & Nayak, 1958), antianaphylactic and antidiarrhoeal (Iyengar & Pendse, 1965), antiamoebic and antiestrogenic (Raghunathan & Mitra, 1982) and antiviral (Dhar et al., 1968) properties. Sharma et al. (1997) reported the hypoglycaemic properties of the seeds of *C. bonducella*, in normal as well as streptozotocin diabetic rats. Recently, the outer shell of the seeds was reported to have hypoglycaemic activity in experimentally induced diabetic animals (Biswas et al., 1997). The seed kernel is used in the management of diabetes mellitus in the folk medicine of the Andaman and Nicobar Islands. Moreover, it is one among the constituents of a decoction used in the Caribbean folk medicine, in the management of diabetes (Peter et al., 1998). Rao et al. (1994) reported the hypoglycaemic effect of the seed kernel powder of *C. bonducella* in alloxan induced diabetic rabbits.

However, a systematic, chronic study, using different extracts of the seed kernels of *C. bonducella* and an attempt to elucidate, its antidiabetic principles was not found. This prompted the present investigators to take up the present study on various extracts of seed kernels of *C. bonducella*. Taking into consideration the role played by oxidative stress in the pathogenesis and progression of diabetes (Baynes, 1991; Hideaki et al., 1999), an attempt was also made in the present study, to find the *in vitro* antioxidant potential of various extracts.

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Materials and methods

Collection of crude drug

The fresh dried seeds of *C. bonducella* was obtained from a local ayurvedic shop at Udupi, Karnataka and was authenticated by Dr. G.K. Bhat, Department of Botany, Poorna Prajna College, Udupi. It was then preserved in the Department of Pharmacology, Kasturba Medical College, Manipal, Karnataka.

Preparation of extracts

The seed kernels were manually separated from the outer seed shell. Then, they were powdered and extracted using petroleum ether (40–60 °C) by Soxhlet extraction. Later, petroleum ether was evaporated off and the residual oil was filtered to obtain petroleum ether extract (P.E. extract). The defatted seed powder was air-dried to remove the solvent and was later subjected to Soxhlet extraction using absolute alcohol (70–80 °C). The alcoholic extract was then obtained after evaporating the alcohol. The alcoholic extract so obtained was further fractionated using solvent ether and ethyl acetate. In both the cases, the respective solvents were evaporated to get the dried extracts (ether extract and ethyl acetate extract) of *C. bonducella*. Aqueous extract (Aq. extract) was obtained by boiling fresh seed powder in distilled water (100 °C) and later by evaporating water from the decanted portion. The four extracts so prepared were subjected for further studies.

A) Chemical studies

Phytochemical investigation of various extracts of *C. bonducella* was conducted for the presence of glycosides (Molisch's test), reducing sugars (Fehling's test; Tollen's reagent test), flavonoids (Shinoda test), saponins (foam test) and triterpinoids (Liebermann-Burchard test).

B) Biological studies

Standard drugs

Alloxan (ALX) used for the induction of diabetes was supplied by Sigma Chemicals, USA. Glibenclamide tablets (Daonil; Hoechst, India) were procured from the hospital pharmacy of Kasturba Medical College, Manipal.

Extract solutions

While an o/w emulsion was prepared with P.E. extract, the remaining extracts were suspended in distilled water with 4% gum acacia, for administration to animals.

Acute toxicity studies

The different extracts of *C. bonducella* were subjected to acute toxicity studies in mice, to determine the safe doses by an up-and-down staircase method as described by Ghosh (1984).

Antidiabetic studies

Experiments were conducted on locally bred, male adult albino rats (150–200 g) of Wistar strain, after obtaining permission from the local ethical committee for animal welfare. The animals were housed individually and maintained on normal standard diet with water *ad libitum*.

i) Studies on normal rats

Sixty rats were divided into six groups of ten animals each. While Group I animals receiving vehicle served as control, Group II to Group VI received P.E. extract (500 mg/kg), ether extract (100 mg/kg), ethyl acetate extract (Et.Ac. extract) (200 mg/kg), Aq. extract (400 mg/kg) and a suspension of glibenclamide (0.5 mg/kg), respectively. While the doses of the extracts were chosen such that they were 1/10th of their safe doses, the dose of glibenclamide was calculated by computing the minimum human dose to rats (Paget & Burnes, 1964). The suitably formulated extracts and glibenclamide were given through the oral route, once a day, for 30 days.

From these animals, upon an overnight fast, blood was withdrawn through the retro orbital plexus, using heparinised haematocrits, on day 30, following 4 h, after administration of vehicle/extracts/standard drug. The fasting blood sugars (FBS) were then estimated using glucose oxidase-peroxidase method (Ranbaxy; Glucose estimation kit).

ii) Studies on ALX-induced diabetic rats

Diabetes was induced in 24 h fasted male adult rats of Wistar strain by a single i.p. injection of alloxan, at a dose of 120 mg/kg b.w. in cold saline (Chattopadhyay et al., 1997). The diabetic rats, after confirmation of stable hyperglycaemia, were then divided into 6 groups of 6–7 rats each. Group I served as the control. The remaining groups received the various extracts and standard drug, glibenclamide, as mentioned above, once a day, for 17 days. From these animals, FBS were determined on two occasions, both zero hour (before administration of drug/extracts) and 4 h after administration, on day 0, day 5, day 10 and day 15. On day 16, the blood samples were withdrawn to determine total cholesterol (TC) and triglyceride (TG) levels (Roche Diagnostics; TC and TG estimation kits). On day 17, all the above groups of animals were sacrificed by single stunning, their livers excised out, weighed and glycogen was estimated using anthrone method (Nicholas, 1956).

C) *In vitro* antioxidant studies

The *in vitro* antioxidant property of various extracts (1 mg/ml) was assessed on the basis of their ability to reduce

the colour intensity produced by the standard free radical, 1,1-diphenyl-2-picryl hydrazyl (DPPH), as described by Kato et al. (1988). The absorbance was read at 517 nm using UV-VIS spectrophotometer (Shimadzu 1201). Percentage scavenging activity of the extracts was calculated as follows:

$$\frac{A_c - A_t}{A_c} \times 100$$

A_c = Absorbance of control (95% ethanol)
A_t = Absorbance of test (extracts)

Percentage scavenging activity vs time course graph was then plotted.

Statistical analysis

Results of biochemical estimations are reported as ±S.E.M. Total variation present in a data was analysed by one way analysis of variance (ANOVA) followed by Scheffe's test using SPSS computer package. For comparing the blood sugar values before and after the drug administration paired Student's *t*-test was employed.

Results

In chemical studies, the aqueous and Et.Ac. extracts showed the presence of glycosides or reducing sugars, saponins and triterpinoids. In the acute toxicity studies, P.E. extract, Aq. extract, ether extract and Et.Ac. extract were found to be safe at the doses, 4.0, 4.0, 2.0 and 1.0 g/kg, respectively. Because of difficulties involved in formulating the extracts beyond the

Table 1. Hypoglycaemic effects of various extracts of *Caesalpinia bonducella* in normal rats.

Groups (n)	Dose (mg/kg)	Fasting blood sugar (30 mg/dl) (FBS)* on day [Mean ± S.E.]
Normal Control (10)	5 ml. of 2% gum acacia	80.72 ± 2.19
P.E. extract (10)	500	77.37 ± 5.2[b]
Ether extract (10)	100	85.62 ± 6.4[b]
Et.Ac. extract (10)	200	68.60 ± 2.0[a,b]
Aq. extract (10)	400	65.80 ± 1.1[a,b]
Glibenclamide (10)	0.5	38.93 ± 2.6[a]
Allowance value by Scheffe's test**		12.0

* Four hours after drug administration.
** SPSS computer package.
a = p < 0.05 Normal Control.
b = p < 0.05 Vs Glibenclamide.
Note: The difference between two means is significant, if the means are differing by the said allowance value.

Table 2. Effect of various extracts of *Caesalpinia bonducella* on FBS in ALX induced diabetic rats.

		FBS (mg/dl) on							
		Day 0		Day 5		Day 10		Day 15	
Groups (n)	Dose (mg/kg)	0th h	4th h	0th h	4th h	0th h	4th h	0th h	4th h
Diabetic control (7)	5 ml. 2% acacia	286.84 ± 31.2	280.0 ± 12.8	277.37 ± 12.9	295.0 ± 21.5	269.26 ± 11.4	280.89 ± 15.9	272.27 ± 8.65	277.77 ± 6.3
P.E. extract (6)	500	236.11 ± 16.7	267.23 ± 19.3	255.79 ± 11.7	298.99 ± 25.9	271.15 ± 9.1	286.37 ± 15.1	275.41 ± 11.4	287.24 ± 10.1
Ether extract (6)	100	248.47 ± 19.4	233.74 ± 16.7[b]	258.56 ± 11.8	251.78 ± 12.3[a]	249.63 ± 11.5	241.34 ± 10.6	239.46 ± 8.8	228.37 ± 10.4[c]
Et.Ac. extract (7)	200	247.67 ± 13.4	221.98 ± 13.8[c]	226.95 ± 14.7	210.24 ± 14.7[c]	213.61 ± 15.3	193.23 ± 16.2[c]	197.23 ± 14.3	179.5 ± 11.9[c]
Aq. extract (7)	400	233.2 ± 10.6	207.65 ± 11.7[b]	236.19 ± 9.69	210.88 ± 12.1[b]	218.36 ± 6.04	190.15 ± 5.41[c]	199.65 ± 11.8	170.4 ± 9.38[c]
Glibenclamide (5)	0.5	241.56 ± 16.9	215.34 ± 14.7[c]	224.42 ± 15.6	205.72 ± 15.0[c]	214.02 ± 14.2	192.45 ± 14.9[c]	195.55 ± 17.1	177.19 ± 17.4[c]

Statistical analysis: Paired Student's *t*-test.
a = p < 0.05 vs Before 0th hour.
b = p < 0.01 vs Before 0th hour.
c = p < 0.001 vs Before 0th hour.

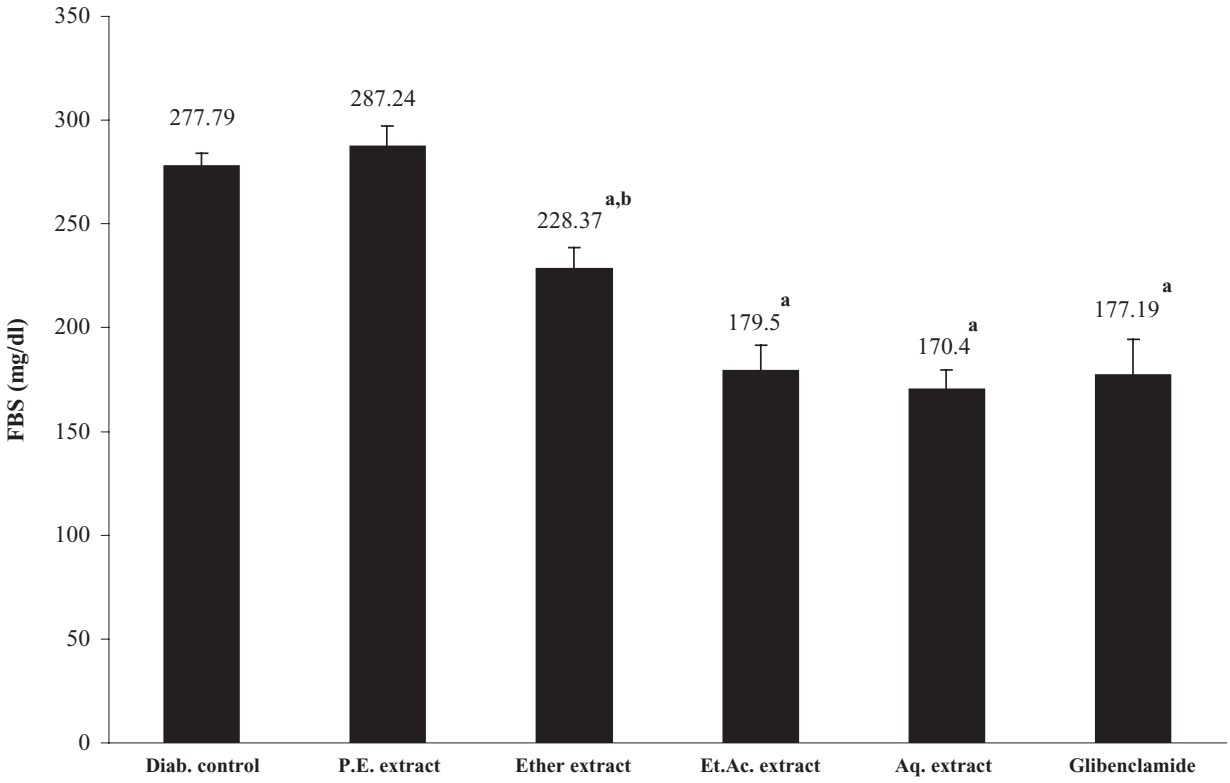

Figure 1. Effect of various extracts of *Caesalpinia bonducella* on FBS in ALX-induced diabetic rats after 15 days of treatment. a = $p < 0.05$ vs Diabetic control; b = $p < 0.05$ vs glibenclamide.

above said doses, the extracts were not tested for their lethal effects in mice. However, at the above said doses, none of the extracts showed any untoward effects on behavioural response, normal reflexes and so on.

In normal rats, only aqueous and Et.Ac. extracts showed a significant ($p < 0.05$) hypoglycaemic effect on day 30 (Table 1). However, as compared to glibenclamide, their effect was significantly less ($p < 0.05$).

In diabetic control animals, a marginal increase in FBS was observed 4 h after vehicle administration, on day 0 and on days, 5, 10 and 15. A similar trend was observed with the P.E. extract as well. Contrary to this, on an average, there was a significant reduction in FBS with extracts such as ether (4%), ethyl acetate (9.2%) and aqueous (12.3%) as well as glibenclamide (9.8%), respectively. Significantly, all these three extracts showed an acute hypoglycaemic effect, similar to that of glibenclamide, on day 0 itself, and continued to maintain this trend till the end of the study (Table 2). On a chronic basis, all the three extracts namely ether, ethyl acetate and aqueous, showed a significant ($p < 0.05$) hypoglycaemic effect as compared to diabetic control (Fig. 1). While the effect of ether extract was inferior to that of glibenclamide, the aqueous and Et.Ac. extracts showed a similar efficacy as that of glibenclamide (Fig. 1).

ALX induced diabetes caused a significant rise in total cholesterol and triglyceride levels as compared to normal animals. The aqueous and Et.Ac. extracts reversed the diabetes induced changes in TC and TG levels, like that of glibenclamide (Table 3). While, in case of other two extracts, ether extract could reverse only TG levels, the P.E. extract had no effect on diabetes induced hyperlipidemia.

ALX induced diabetes caused a significant depletion of glycogen stores in the liver. Aq. extract and Et.Ac. extract were found to restore the liver glycogen stores in the liver and their effect was comparable to that of glibenclamide (Table 3).

In antioxidant studies, while Aq. extract did not show any free radical scavenging activity, the remaining three extracts, namely, ethyl acetate, ether and petroleum ether, showed 49, 42 and 31% free radical scavenging activity at the end of 60 min (Fig. 2).

Discussion

The present study was undertaken with the objective of discovering the antidiabetic principles of various extracts of *Caesalpinia bonducella*, a herb which has been widely used in the folk medicine of Andaman and Nicobar islands, as well as the Caribbean islands.

The study revealed that the most active antidiabetic principles of the plant were present in the extracts of aqueous

Table 3. Effect of various extracts of *Caesalpinia bonducella* on lipid profiles and liver glycogen in ALX induced diabetic rats.

Groups (n)	Dose (mg/kg)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Liver glycogen (mg/g) ±S.E.M.
Normal control (9)	5 ml. 2% acacia	67.22 ± 2.87	47.33 ± 2.36	3.86 ± 0.56
Diabetic control (7)	5 ml. 2% acacia	96.28 ± 3.61[a]	100.85 ± 6.29[a]	1.38 ± 0.13[a]
P.E. extract (6)	500	84.0 ± 4.9[a]	98.66 ± 5.55[a]	1.29 ± 0.15[a]
Ether extract (6)	100	71.16 ± 3.3[b]	85.16 ± 3.46[a]	1.57 ± 0.13[a]
Et.Ac. extract (7)	200	63.42 ± 2.1[b]	63.0 ± 2.42[b]	2.55 ± 0.2
Aq. extract (7)	400	66.57 ± 1.88[b]	65.57 ± 3.59[b]	3.35 ± 0.19[b]
Glibenclamide (5)	0.5	70.6 ± 2.42[b]	70.2 ± 3.42[a,b]	3.5 ± 0.21[b]
Allowance value by Scheffe's test**	–	17.0	21.0	1.78

a = $p < 0.05$ vs Normal control.
b = $p < 0.05$ vs Diabetic control.
** SPSS Computer package.
Note: The difference between two means is significant, if the means differ by the said allowance values.

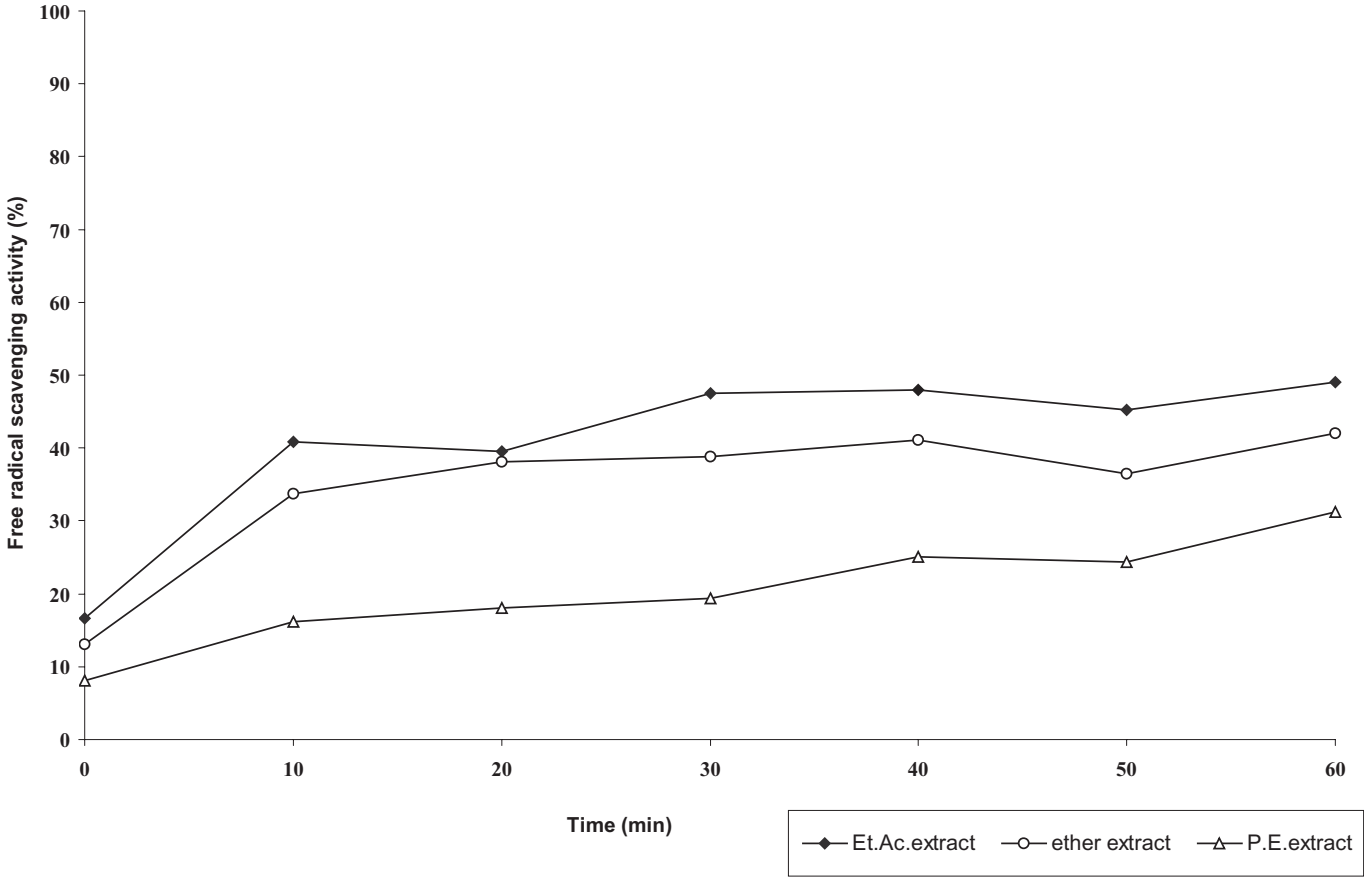

Figure 2. Chart showing the DPPH free radical scavenging activity of various extracts at different time intervals.

and ethyl acetate. These two extracts showed a significant hypoglycaemic effect in normal as well as ALX induced diabetic rats. Besides, in ALX-induced diabetic model, these two extracts could reverse, the diabetes induced changes in lipid profiles and liver glycogen, the effects of which were comparable to that of glibenclamide.

While these two active extracts have a minimum hypoglycaemic effect in normal rats, they were equi-efficacious to that of glibenclamide in ALX-induced diabetic rats. The reason for their low hypoglycaemic activity in normoglycaemic conditions may be due to their inability to disturb the carbohydrate homeostasis maintenance. However, in the ALX-induced diabetic model, they could have acted in a manner similar to that of glibenclamide, that is, releasing insulin from the surviving beta cells of pancreas. These findings corroborate earlier reports on the hypoglycaemic potential of outer shell of seeds of *C. bonducella* (Biswas et al., 1997). From the results of the present study, it is very difficult to predict the exact mechanism for this differential action.

From the results of the present study, it is evident that the highly polar extracts of seed kernels of *C. bonducella*, namely aqueous and ethyl acetate, possess the most active antidiabetic principles of the plant. This is because the non-polar extracts such as ether, showed poor antidiabetic activity and the P.E. extract failed to show any activity. Since both the active extracts were, chemically, found to contain triterpinoidal glycosides, we presume that they might be the active principles contributing to the antidiabetic actions. This is further supported by various reports on the hypoglycaemic potential of triterpenoids in *Panax ginseng* Mey. (ginsenoside), *Cornus officinalis* Linn. (ursolic acid), *Momordica cochinchinensis* Spreng. (oleanolic acid) and so on (Ivorra et al., 1989).

From the results of *in vitro* antioxidant studies, it is clear that the antioxidant potentials of extracts need not necessarily contribute to their hypoglycaemic potential. This is because of the fact that out of two polar active extracts, namely, aqueous and ethyl acetate, the former was devoid of any antioxidant activity, while the latter had the highest antioxidant activity. On the other hand, the P.E. extract failed to show any hypoglycaemic effect in spite of 31% free radical scavenging activity. However, the antioxidant potential of ethyl acetate extract of seed kernels of *C. bonducella* could contribute very well to its antidiabetic potential, by minimising the diabetes associated oxidative stress linked complications such as retinopathy, cardiac myopathy, atherosclerosis, nephropathy and so on (Baynes, 1991; Sato et al., 1979; Larkins et al., 1992).

In conclusion, it could be said that the claims on *Caesalpinia bonducella*, for its antidiabetic activity, are vindicated. Considering the fact that the active extracts are very safe, even at high doses, its antidiabetic potential might play an adjuvant role in the management of diabetes mellitus.

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